GEOMICROBIOLOGY

Fourth Edition, Revised and Expanded

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To my former students,
from whom I have learned as much
as I hope they have learned from me.

Preface to the Fourth Edition

The field of geomicrobiology has been receiving wider recognition than ever before among environmental microbiologists and earth scientists since the first appearance of the third edition of this book in November 1995. This is happening because of an ever-increasing awareness of the influence of microbial activity in shaping the habitable part of our planet. The pace of research on various aspects of geomicrobiology in the last few years has significantly accelerated and produced new discoveries of geomicrobial phenomena and yielded new insights into previously established phenomena. The topic of geomicrobiology was specifically addressed in the program of recent annual meetings of the American Society of Microbiology (ASM) and, since the year 2000, has been allotted a special section in the table of contents of the journal *Applied and Environmental Microbiology*, published by ASM. A journal exclusively devoted to the subject, the *Geomicrobiology Journal*, has been published independently of ASM since 1978. The timely publication of *Geomicrobiology: Fourth Edition, Revised and Expanded*, puts the new advances in the field in perspective.

This fourth edition incorporates the important new findings of geomicrobial significance of the last five years. Some of these findings were made by the

application of new physical and biological analytical techniques. They enlarged our concept of the total size of the microbial habitat enormously because living microorganisms have been detected below the Earth's surface at significantly greater depths than heretofore. They have also expanded our understanding of the great diversity among the microbes in all the habitable parts of the Earth. Intensive investigations are ongoing to determine the interrelationships among the microorganisms in these habitats and the nature of their activities from a geomicrobial standpoint. This edition reflects some modifications in the thinking about the origin of life on Earth and its early evolution, but a divergence of views remains.

In regard to specific geomicrobial processes, the fourth edition reflects the increase in our understanding of the microbial weathering of rocks and minerals. It contains a new chapter that deals with a probable role of microbes in the formation of bauxites. The chapters on iron and manganese incorporate the latest findings in regard to the physiology of microbial oxidation and reduction of ionic forms of these metals and some of their minerals and the diversity of the organisms involved. They also contain a more extensive discussion of the microbial role in anaerobic biodegradation of organic carbon than in earlier editions. The chapters dealing with sulfur, arsenic, and selenium compounds incorporate the latest findings with regard to microbial oxidation and/or reduction of corresponding forms of these elements. The section on microbial metal sulfide oxidation in Chapter 19 has been extensively modified. It reflects the important recent discovery that acidophilic *Thiobacillus ferrooxidans* (recently renamed *Acidithiobacillus ferrooxidans*) seems to be a secondary rather than a primary player in mobilizing metal from metal sulfide ores in heap-, dump-, and in situ leaching and in generating acid mine drainage, at least in more advanced stages. This chapter also examines a current controversy concerning the mechanism by which acidophilic iron bacteria oxidize metal sulfides. New references have been added to the chapter on the geomicrobiology of fossil fuels.

The chief aim of the fourth edition of *Geomicrobiology*, like that of the earlier editions, is to serve as an introduction to the subject and to be of use as a text as well as an up-to-date reference book. The book includes discussion of the older literature as well as the recent literature, which is important for an appreciation of the development of the different areas of geomicrobiology. As in the earlier editions, the reference lists at the end of each chapter are not exhaustive but include the literature I deem most important. Related literature can be located by cross-referencing. As in previous editions, a glossary is included to provide definitions of scientific terms that may be unfamiliar to some readers.

I have retained some of the drawings prepared by Stephen Chiang for the first edition. A few illustrations from the third edition have been replaced, and a few entirely new ones have been added. I am indebted to a number of persons and publishers for making available original photographs or allowing reproduction of

previously published material. They are acknowledged in the legends of the individual illustrations.

I owe thanks to Donna Bedard for very helpful comments on the molecular aspects discussed in Chapter 7. My thanks also go to Jill Banfield, Katarina Edwards, and Francisco F. Roberto for reading Chapter 19, and to Ronald Oremland for reading Chapters 13 and 20. I am indebted to Sigal Lechno-Yossef for help with the digital photomicrography setup in the biology department.

The continued belief of Marcel Dekker, Inc., in the importance of this book has encouraged me greatly in preparing this fourth edition. Special thanks go to Sandra Beberman, Vice President, Medical Division; Michael Deters and Moraima Suarez, Production Editors; and the editorial staff.

Responsibility for the presentation and interpretation of the subject matter in this edition rests entirely with me.

Henry Lutz Ehrlich

Preface to the Third Edition

The need for a third edition of *Geomicrobiology* has arisen because of some important advances in the field since the second edition. Of particular significance are advances in the areas of subsurface microbiology as it relates to groundwater, carbonate deposition, rock weathering, methylmercury formation, oxidation and reduction of iron and manganese, chromate reduction, oxidation and reduction of molybdenum, reduction of vanadate (V) and uranium (VI), oxidation and reduction of sulfur compounds, reduction of selenate and selenite, methanogenesis, microbial attack of coal, and degradation of hydrocarbons. These advances have been integrated into the treatment of these subjects. The chapter dealing with the biochemistry and physiology of geomicrobial processes has been updated to convey the basis for our current understanding of how and why microbes are involved in these processes.

Because this book is meant to serve as a reference as well as a textbook, very little material from the second edition has been eliminated. By retaining this information, an overview of the growth of the field of geomicrobiology since its inception is retained. It enables newcomers to learn what has been accomplished in the field and to gain an introduction to the literature. The literature citations on

the different subjects are not exhaustive, but include the most important ones, making it possible to locate other works by cross-referencing. As in the previous editions, a glossary is included to aid in the definition of unfamiliar scientific terms.

In preparing this edition, I have retained some of the line drawings prepared by Stephen Chiang for the first edition that were also included in the second edition. Some other illustrations from the second edition have been replaced, and a few entirely new illustrations have been included. I am indebted to a number of persons and publishers for making available original photographs or allowing reproduction of previously published material. They are acknowledged in the legends of the individual illustrations.

I wish to thank Marcel Dekker, Inc., for their continued belief in the importance of this book by encouraging the preparation of this third edition. I want to express special thanks to Bradley Benedict, Assistant Production Editor, and the editorial staff for their assistance in preparing this edition.

Responsibility for the presentation and interpretation of the subject matter in this edition rests entirely with me.

Henry Lutz Ehrlich

Preface to the Second Edition

As in the first edition of this book, geomicrobiology is presented as a field distinct from microbial ecology and microbial biogeochemistry. The stress remains on examination of specific geomicrobial processes, microorganisms responsible for them, and the pertinence of these processes to geology.

Most chapters from the earlier edition have been extensively revised and updated. As far as possible, new discoveries related to geomicrobiology reported by various investigators since the writing of the first edition have been integrated into the new edition. Two new chapters have been added, one on the geomicrobiology of nitrogen and the other on the geomicrobiology of chromium. The second chapter of the first edition has been divided into two to allow for a more concise development of the two topics: Earth as microbial habitat and the origin of microbial life on Earth.

In the new edition, Chapters 2–6 are intended to provide the background needed for understanding the succeeding chapters, which deal with specific aspects of geomicrobiology. An understanding of microbial physiology and biochemistry is very important for a full appreciation of how specific microbes

can act as geomicrobial agents. For this reason, Chapter 6 was extensively revised from its antecedent, Chapter 5, in the first edition.

Like its predecessor, the present edition is meant to serve not only as a text, but also as a general introduction and guide to the geomicrobial literature for microbiologists, ecologists, geologists, environmental engineers, mining engineers, and others interested in the subject. The literature citations are not intended to be exhaustive, but cross-referencing, especially in cited review articles, should lead the reader to many other pertinent references not mentioned in this book.

Some of the revisions in this edition, especially those relating to bioenergetics, were significantly influenced by a number of stimulating informal discussions with my colleague and research collaborator John C. Salerno.

In preparing this edition, I have retained some of the line drawings by Stephen Chiang. I have, however, replaced many of the other illustrations, and added some new ones that I prepared on a Macintosh Plus computer with Cricket Draw and Cricket Graph applications. I wish to thank the Voorhees Computer Center of Rensselaer Polytechnic Institute for allowing me to use the Laser Printer Facility and George Clarkson for making the necessary arrangements. Once again, I am indebted to a number of persons and publishers for making available original photographs or allowing reproduction of previously published material. They are acknowledged in the legends of the individual illustrations.

I wish to thank Marcel Dekker, Inc., for deeming the subject matter of this book of sufficient continued importance to publish this second edition. Special thanks go to Judith DeCamp, Production Editor, and the editorial staff for their help in bringing this edition to fruition.

Responsibility for the presentation and interpretation of the subject matter in this edition rests entirely with me.

Henry Lutz Ehrlich

Preface to the First Edition

This book deals with geomicrobiology as distinct from microbial ecology and microbial biogeochemistry. Although these fields overlap to some degree, each emphasizes different topics (see Chapter 1). A reader of this book should not, therefore, expect to find extensive discussions of ecosystems, food chains, nutritional cycles, mass transfer, or man-made pollution problems as such, because these topics are not at the heart of geomicrobiology. Geomicrobiology is the study of the role that microbes play or have played in specific geological processes.

This book arose out of a strong need I felt in teaching a course in geomicrobiology. As of this writing, no single text is available that deals with the group of topics presented in this book. Previously, students in my geomicrobiology course needed to be referred to the many primary publications on the various topics. These publications are very numerous and are scattered among a plethora of journals and books that are often not readily available. Some are written in languages other than English. This book is an attempt to glean the basic geomicrobial principles from this literature and to illustrate these principles with many different examples.

Some readers of this book will have a stronger background in Earth and marine science than in microbial physiology, while others will have a stronger background in microbial physiology than in Earth and marine sciences. To enable all these readers to place the geomicrobial discussions in the later chapters in proper context, the introductory Chapters 2–5 were written. They are not meant to be definitive treatises on their subjects, and as a result any one of them will appear elementary to a person already knowledgeable in its field. However, I have found the material in these chapters to be essential in teaching my students.

As for the rest of the book, Chapter 6 summarizes the methods used in geomicrobiology, and Chapters 7–17 examine specific geomicrobial activities in relation to geologically important classes of substance or elements. A single basic theme pervades these last 11 chapters: biooxidation and bioreduction and/or bioprecipitation and biosolution. This may seem an unnecessary reiteration of a common set of principles, but closer examination will show that the manifestations of these principles in different geomicrobial phenomena differ so strikingly as to require separate examination. In discussing geomicrobial processes, I have tended to emphasize the physiological more than the geological aspects. This is in part because the former is my own area of greater expertise, but also, and more importantly, because I feel that the physiological and biochemical nature of geomicrobial processes has to be understood to fully appreciate why some microbes are capable of these activities.

In citing microorganisms in the text, the names employed by the investigators whose work is described are used. In the case of bacteria, these names may have subsequently changed. The currently used names of the bacteria may be found by referring to *Bergey's Manual of Determinative Bacteriology* (8th edition, edited by R. E. Buchanan and N. E. Gibbons, 1974, Williams and Wilkins, Baltimore) and to the *Index Bergeyana* (R. E. Buchanan, J. G. Holt, and E. F. Lessel, 1966, Williams and Wilkins, Baltimore). In some instances, however, it may be impossible to find a bacterial organism listed in the *Manual* or the *Index* because the organism was never sufficiently described to achieve taxonomic status. The current names of renamed bacteria may also be found in the index of organisms at the end of this book.

It is hoped that this book will serve not only as a text but also as an introduction and guide to the geomicrobiological literature for microbiologists, ecologists, geologists, environmental engineers, and others interested in the subject.

The preparation of this book was greatly aided by discussion with, and review of the manuscript by, Galen E. Jones, R. Schweisfurth, William C. Ghiorse, Edward J. Arcuri, Paul A. LaRock, and many students in my geomicrobiology course. Responsibility for the presentation and interpretation of the subject matter as found in this book rests, however, entirely with me. I am indebted to a number of persons and publishers for making available original

photographs or allowing reproduction of previously published material for illustration. They are acknowledged in the legends of the individual illustrations. I wish to thank Stephen Chiang for his preparation of finished line drawings from the crude sketches I furnished. I also wish to thank the editorial staff of Marcel Dekker, Inc., for their help in readying my manuscript for publication.

Henry Lutz Ehrlich

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1

Introduction

Geomicrobiology examines the role that microbes have played in the past and are currently playing in a number of fundamental geological processes. Examples of such processes are the weathering of rocks, soil and sediment formation and transformation, the genesis and degradation of minerals, and the genesis and degradation of fossil fuels. Geomicrobiology should not be equated with microbial ecology or microbial biogeochemistry. *Microbial ecology* is the study of interrelationships between different microorganisms; among microorganisms, plants, and animals; and between microorganisms and their environment. *Microbial biogeochemistry* is the study of microbially influenced geochemical reactions, enzymatically catalyzed or not, and their kinetics. These reactions are often studied in the context of mineral cycles, with emphasis on environmental mass transfer and energy flow. These three subjects do overlap to some degree, as shown in Figure 1.1.

The origin of the word "geomicrobiology" is obscure. It obviously derived from the term "geological microbiology." Beerstecher (1954) defined geomicrobiology as "the study of the relationship between the history of the Earth and microbial life upon it." Kuznetsov et al. (1963) defined it as "the study of microbial processes currently taking place in the modern sediments of various bodies of water, in ground waters circulating through sedimentary and igneous rocks, and in weathered Earth crust [and also] the physiology of specific

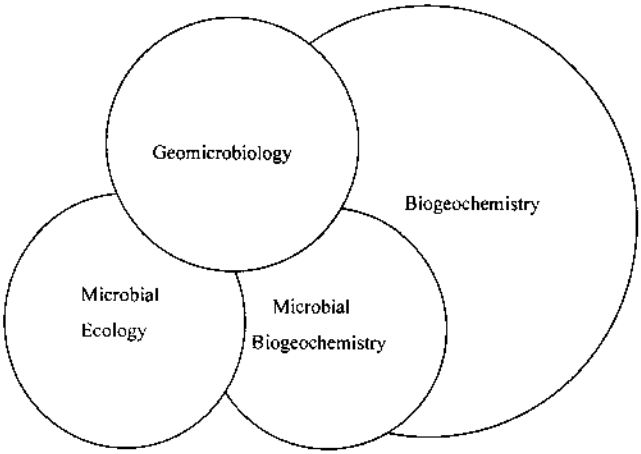


FIG. 1.1 Interrelationships among geomicrobiology, microbial ecology, microbial biogeochemistry, and biogeochemistry.

microorganisms taking part in presently occurring geochemical processes." Neither author traced the history of the word, but they pointed to the important roles that scientists such as Winogradsky, Waksman, and ZoBell played in the development of the field.

Geomicrobiology is not a new field, although until the last few years it did not receive much attention. Certain early investigators in soil and aquatic microbiology may not have thought of themselves as geomicrobiologists, but they nevertheless had an influence on the subject. One of the first contributors to geomicrobiology was Ehrenberg (1838), who in the second quarter of the nineteenth century discovered the association of *Gallionella ferruginea* with ochreous deposits of bog iron. He believed that the organism, which he thought to be an infusorian but which we now recognize as a stalked bacterium, was important in the formation of such deposits. Another important early contributor to geomicrobiology was Winogradsky, who discovered that *Beggiatoa* could oxidize H_2S to elemental sulfur (1887) and that *Leptothrix ochracea* promoted oxidation of $FeCO_3$ to ferric oxide (1888). He believed that both organisms gained energy from these processes. Still other important early contributors to geomicrobiology were Harder (1919), a researcher trained as a geologist and microbiologist, who studied the significance of microbial iron oxidation and precipitation in relation to the formation of sedimentary iron deposits, and Stutzer (1912), Vernadsky (1908–1922)(1955), and others, whose studies led to recognition of the significance of microbial oxidation of H_2S to elemental sulfur in the formation of sedimentary sulfur deposits [see Ivanov (1967), Lapo (1987), and

Bailes (1990) for a discussion of early Russian geomicrobiology and its practitioners]. Our understanding of the role of bacteria in sulfur deposition in nature received a further boost from the discovery of bacterial sulfate reduction by Beijerinck (1895) and van Delden (1903).

Starting with the Russian investigator Nadson (1903) (see also Nadson, 1928) at the end of the nineteenth century, and continuing with such investigators as Bavendamm (1932), the important role of microbes in $CaCO_3$ precipitation began to be noted. Microbial participation in manganese oxidation and precipitation in nature was first indicated by Beijerinck (1913), Soehngen (1914), Lieske (1919), and Thiel (1925). Zappfe (1931) later related this activity to the formation of sedimentary manganese ore. The microbial role in methane formation became apparent through the observations and studies of Béchamp (1868), Tappeiner (1882), Popoff (1875), Hoppe-Seyler (1886), Omeliansky (1906), and Soehngen (1906); see also Barker (1956). The role of bacteria in rock weathering was first suggested by Muentz (1890) and Merrill (1895). Later, involvement of acid-producing microorganisms such as nitrifiers and of crustose lichens and fungi was suggested (see Waksman, 1932). Thus by the beginning of the twentieth century, many of the important areas of geomicrobiology had begun to receive serious attention from microbiologists. In general, it may be said that most of the geomicrobiologically important discoveries of the nineteenth century were made through physiological studies in the laboratory that revealed the capacity of specific organisms for geomicrobiologically important transformations, causing later workers to study the extent of the microbial activities in the field.

Geomicrobiology in the United States can be said to have begun with the work of E. C. Harder (1919) on iron-depositing bacteria. Other early American investigators of geomicrobial phenomena include J. Lipman, S. A. Waksman, R. L. Starkey, and H. O. Halvorson, all prominent in soil microbiology, and G. A. Thiel, C. Zappfe, and C. E. ZoBell, all prominent in aquatic microbiology. ZoBell was a pioneer in marine microbiology (Ehrlich, 2000).

Very fundamental discoveries in geomicrobiology continue to be made, some basic ones having been made as the twentieth century progressed and others very recently. For instance, the concept of environmental limits of pH and E_h for microbes in their natural habitats was first introduced by Baas Becking et al. (1960) (see Chap. 6). Life at high temperature in nature was systematically studied for the first time in the 1970s by Brock and associates in Yellowstone Park, in the United States (Brock, 1978). A specific acidophilic, iron-oxidizing bacterium and its association with the production of acid coal mine drainage was first discovered in the late 1940s, the result of studies by Colmer et al. (1950) (see Chaps. 15 and 19). The subsequent demonstration of the presence of these same organisms in acid mine drainage from a copper sulfide ore body in Utah (Bingham Canyon open pit mine) and the experimental finding that these organisms can promote the leaching of metals from various metal sulfide ores

(Bryner et al., 1954) led to the first industrial application of geomicrobially active organisms (Zimmerley et al., 1958) (Chap. 19). The first attempts at visual detection of Precambrian prokaryotic fossils in sedimentary rocks were made by Tyler and Barghoorn in 1954 and by Schopf et al. and by Barghoorn and Schopf in 1965 (see Chap. 3). Paleontological discoveries resulting from these studies have had a profound influence on current theories about the evolution of life on Earth (Schopf, 1983). The discovery of geomicrobially active microorganisms around submarine hydrothermal vents (Jannasch and Mottl, 1985; Tunnicliffe, 1992) and the demonstration of a significant viable microflora with the potential for geomicrobially important activity in the deep subsurface of continents (Ghiorse and Wilson, 1988; Sinclair and Ghiorse, 1989; Fredrickson et al., 1989; Pederson, 1993) and the ocean floor (Parkes et al., 1994) are opening up previously unsuspected new topics for geomicrobial study.

As this book will show, many areas of geomicrobiology remain to be fully explored or developed further.

REFERENCES

Baas Becking LGM, Kaplan IR, Moore D. 1960. Limits of the environment in terms of pH and oxidation and reduction potentials. J Geol 68:243–284.

Bailes KE. 1990. Science and Russian Culture in an Age of Revolution. V. I. Vernadsky and His Scientific School, 1863–1945. Bloomington: Indiana Univ Press.

Barghoorn ES, Schopf JW. 1965. Microorganisms from the late Precambrian of Central Australia. Science 150:337–339.

Barker HA. 1956. Bacterial Fermentations. CIBA Lectures Microb Biochem. New York: Wiley.

Bavendamm W. 1932. Die mikrobiologische Kalkfällung in der tropischen See. Arch Mikrobiol 3:105–276.

Béchamp E. 1868. Ann Chim Phys 13:103 (as cited by Barker, 1956).

Beerstecher E. 1954. Petroleum Microbiology. New York: Elsevier.

Beijerinck MW. 1895. Über *Spirillum desulfuricans* als Ursache der Sulfatreduktion. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt I Orig 1:1–9, 49–59, 104–114.

Beijerinck MW. 1913. Oxydation des Mangancarbonates durch Bakterien und Schimmelpilzen. Folia Microbiol (Delft) 2:123–134.

Brock TD. 1978. Thermophilic Microorganisms and Life at High Temperatures. New York: Springer-Verlag.

Bryner LC, Beck JV, Davis DB, Wilson DG. 1954. Microorganisms in leaching sulfide minerals. Ind Eng Chem 46:2587–2592.

Colmer AR, Temple KL, Hinkle HE. 1950. An iron-oxidizing bacterium from the acid drainage of some bituminous coal mines. J Bacteriol 59:317–328.

Ehrenberg CG. 1838. Die Infusionsthierchen als vollkommene Organismen. Leipzig, Germany: L Voss.

Ehrlich HL. 2000. ZoBell and his contributions to the geosciences. 2000. In: Bell CR, Brylinsky M, Johnson-Green P, eds. Microbial Biosystems: New Frontiers. Proc 8th Int

Symp Microb Ecol. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, vol. 1, pp 57–62.
Fredrickson JK, Garland TR, Hicks RJ, Thomas JM, Li SW, McFadden K. 1989. Lithotrophic and heterotrophic bacteria in deep subsurface sediments and their relation to sediment properties. Geomicrobiol J 7:53–66.
Ghiorse WC, Wilson JT. 1988. Microbial ecology of the terrestrial subsurface. Adv Appl Microbiol 33:107–172.
Harder EC. 1919. Iron depositing bacteria and their geologic relations. US Geol Surv Prof Pap 113.
Hoppe-Seyler FZ. Physiol Chem 1886. 10:201, 401 (as cited by Barker, 1956).
Ivanov MV. 1967. The development of geological microbiology in the U.S.S.R. Mikrobiologiya 31:795–799.
Jannasch HW, Mottl MJ. 1985. Geomicrobiology of the deep sea hydrothermal vents. Science 229:717–725.
Kuznetsov SI, Ivanov MV, Lyalikova NN. 1963. Introduction to Geological Microbiology. English transl. New York: McGraw-Hill.
Lapo AV. 1987. Traces of Bygone Biospheres. Moscow: Mir Publishers.
Lieske R. 1919. Zur Ernährungsphysiologie der Eisenbakterien. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt II 49:413–425.
Merrill GP. 1895. Geol Soc Am Bull 6:321–332 (as cited by Waksman, 1932).
Muentz A. 1890. Sur la décomposition des roches et la formation de la terre arable. CR Acad Sci (hébd séances) (Paris) 110:1370–1372.
Nadson GA. 1903. Microorganisms as Geologic Agents. I. Tr Komisii Isslect Min Vodg St Petersburg: Slavyanska, 1903.
Nadson GA. 1928. Beitrag zur Kenntnis der bakteriogenen Kalkablagerung. Arch Hydrobiol 19:154–164.
Omeliansky W. 1906. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt II 15:673 (as cited by Barker, 1956).
Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightman AJ, Harvey SM. 1994. Deep bacterial biosphere in Pacific Ocean sediments. Nature (Lond) 371:410–413.
Pedersen K. 1993. The deep subterranean biosphere. Earth-Sci Rev 34:243–260.
Popoff L. 1875. Arch Ges Physiol 10:142 (as cited by Barker, 1956).
Schopf JW, ed. 1983. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press.
Schopf JW, Barghoorn ES, Maser MD, Gordon RO. 1965. Electron microscopy of fossil bacteria two billion years old. Science 149:1365–1367.
Sinclair JL, Ghiorse WC. 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol J 7:15–31.
Soehngen NL. 1906. Het oustaan en verdwijnen van waterstof en methaan ouder invloed van het organische leven. Thesis. Technical Univ, Delft. Delft, Netherlands: Vis, Jr.
Soehngen NL. 1914. Umwandlung von Manganverbindungen unter dem Einfluss mikrobiologischer Prozesse. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt II 40:545–554.
Stutzer O. 1912. Origin of sulfur deposits. Econ Geol 7:733–743.

Tappeiner W. 1882. Ber Deut Chem Ges 15:999 (as cited by Barker, 1956).
Thiel GA. 1925. Manganese precipitated by microorganisms. Econ Geol 20:301–310.
Tunnicliffe V. 1992. Hydrothermal-vent communities of the deep sea. Am Sci 80:336–349.
Tyler SA, Barghoorn ES. 1954. Occurrence of structurally preserved plants in Precambrian rocks of the Canadian Shield. Science 119:606–608.
van Delden A. 1903. Beitrag zur Kentnis der Sulfatreduktion durch Bakterien. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt II 11:81–94.
Vernadsky VI. 1955. An Attempt at Descriptive Mineralogy. Izbrannye Trudy, Vol 2. Izdatel'stvo. Moscow: Akad Nauk SSSR.
Waksman SA. 1932. Principles of Soil Microbiology. 2nd ed rev. Baltimore, MD: William & Wilkins.
Winogradsky S. 1887. Über Schwefelbakterien. Bot Ztg 45:489–600.
Winogradsky S. 1888. Über Eisenbakterien. Bot Ztg 46:261–276.
Zappfe C. 1931. Deposition of manganese. Econ Geol 26:799–832.
Zimmerley SR, Wilson DG, Prater JD. 1958. Cyclic leaching process employing iron oxidizing bacteria. US Patent 2,829,964.

2

The Earth as a Microbial Habitat

2.1 GEOLOGICALLY IMPORTANT FEATURES

The interior of the planet Earth consists of three successive regions (Fig. 2.1), the innermost being the *core*. It is surrounded by the *mantle*, which, in turn, is surrounded by the outermost region, the *crust*. The crust is surrounded by a gaseous envelope, the **atmosphere**.

The core, whose radius is estimated to be about 3450 km, is believed to consist of an Fe-Ni alloy with an admixture of small amounts of the siderophile elements cobalt, rhenium, and osmium, very probably some sulfur and phosphorus, and perhaps even hydrogen (Mercy, 1972; Anderson, 1992; Wood, 1997). The inner portion of the core, which has an estimated radius of about 1250 km, is solid, having a density of 13 g cm^{-3} and being subjected to a pressure of 3.7×10^{12} dyn cm^{-2}. The outer portion of the core has a thickness of about 2200 km and is molten, owing to the higher temperature but lower pressure than at the central core ($1.3–3.2 \times 10^{12}$ dyn cm^{-2}). The density of this portion is 9.7–12.5 g cm^{-3}.

The mantle, which has a thickness of about 2865 km, has a very different composition from the core and is separated from it by the Wickert–Gutenberg discontinuity (Madon, 1992). Seismic measurements of the mantle regions have revealed distinctive regions called the upper mantle (365 km thick), transition zone (270 km thick), and lower mantle (1230 km thick) (Madon, 1992). The

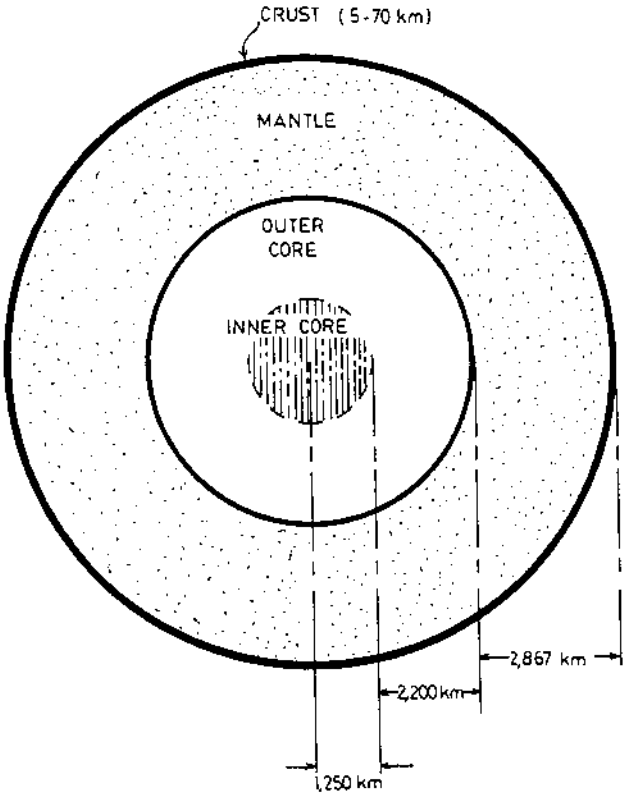


FIG. 2.1 Diagrammatic cross section of the Earth. Radii of core and mantle drawn to scale.

mantle rock is dominated by the elements O, Mg, and Si with lesser amounts of Fe, Al, Ca, and Na (Mercy, 1972). The consistency of the rock in the upper mantle, although not truly molten, is thought to be plastic, especially in the region called the **asthenosphere**, situated 100–220 km below the Earth's surface (Madon, 1992). Upper mantle rock penetrates the crust on rare occasions and may be recognized as an outcropping, as in the case of some ultramafic rock on the bottom of the western Indian Ocean (Bonatti and Hamlyn, 1978).

The crust is separated from the mantle by the Mohorovičic discontinuity. The thickness of the crust varies from as little as 5 km under ocean basins to as great as 70 km under continental mountain ranges. The average crustal thickness is 45 km (Madon, 1992; Skinner et al., 1999). The rock of the crust is dominated by O, Si, Al, Fe, Mg, Na, and K (Mercy, 1972). These elements make up 98.03%

of the weight of the crust (Skinner et al., 1999) and occur predominantly in the rocks and sediments. The bedrock under the oceans is generally basaltic, whereas that of the continents is granitic to an average crustal depth of 25 km. Below this depth it is basaltic to the Mohorovičic discontinuity (Ronov and Yaroshevsky, 1972, p. 243). Sediment covers most of the bedrock under the oceans. It ranges in thickness from 0 to 4 km. Sedimentary rock and sediment (soil in a nonaquatic context) cover the bedrock of the continents; their thickness may exceed that of marine sediments (Kay, 1955, p. 655). The continents make up 64% of the crustal volume, the oceanic crust makes up 21%, and the shelf and subcontinental crust make up the remaining 15% (Ronov and Yaroshevsky, 1972).

Although until the 1960s it was usually viewed as a coherent structure that rests on the mantle, the Earth's crust is now seen to consist of a series of moving and interacting *plates* of varying sizes and shapes. Some plates support the continents and parts of the ocean floor, whereas others support only parts of the ocean floor. The present estimate of the number of major plates involved is still not fully agreed upon but ranges from 10 to 12 (Keary, 1993) to 16 according to the National Geographic Society (1995). Figure 2.2 shows the outlines of some of the major plates and adjacent continents. The plates float on the asthenosphere of the mantle. The crust plus the upper mantle above the asthenosphere is sometimes referred to as the *lithosphere* by geologists. Convection resulting from the thermal gradients in the plastic rock of the asthenosphere is believed to be the cause of movement of the crustal plates (e.g., Kerr, 1995; Wysession, 1995; Ritter, 1999). In some locations this movement may manifest itself in collision of plates, in

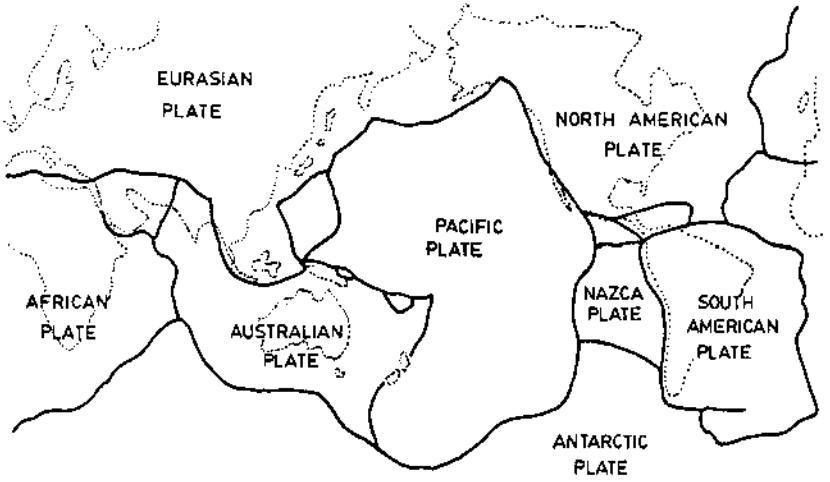


FIG. 2.2 Major crustal plates of the Earth.

other locations in a sliding past each other along transform faults, and in still other locations in sliding over each other. The last process is called **subduction** (crustal convergence). It may result when a denser oceanic plate slides below a lighter continental plate, or when adjacent oceanic plates of nearly equal density interact. Either interaction may lead to formation of a trench–volcanic island arc system. In the case of oceanic–continental plate collisions, the resulting arc system may eventually accrete to the continental margin as a result of the movement of the subducting oceanic plate in the direction of the continental plate. The island arc system results from a sedimentary wedge formed by the oceanic plate (Van Andel, 1992; Gurnis, 1992).

Oceanic plates grow along oceanic ridges, the sites of *crustal divergence*. Examples are the Mid-Atlantic Ridge and the East Pacific Rise (Fig. 2.3). The oldest portions of growing oceanic plates are destroyed through subduction with the formation of deep-sea trenches, such as the Marianas, Kurile, and Philippine trenches in the Pacific Ocean and the Puerto Rico Trench in the Atlantic Ocean. Growth of the oceanic plates at the mid-ocean ridges is the result of submarine volcanic eruptions of **magma** (molten rock from the deep crust or upper mantle). This magma is added to opposing plate margins along a mid-ocean ridge, causing

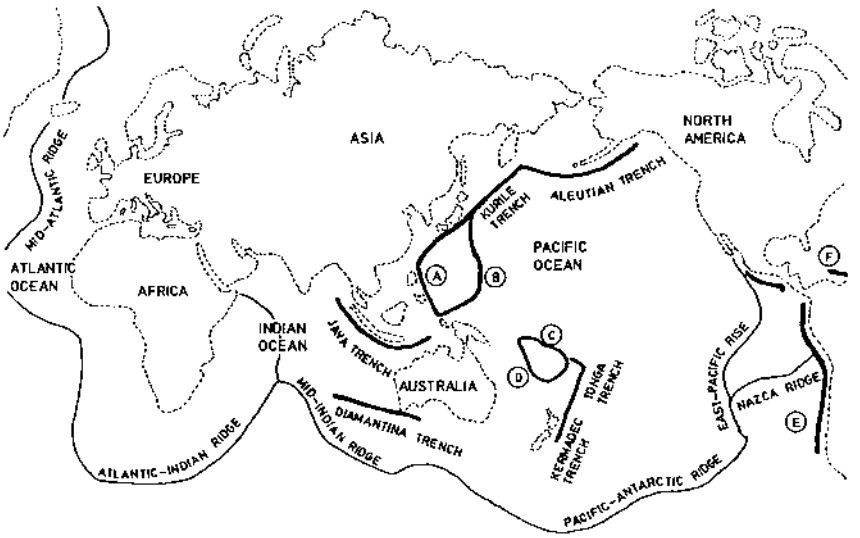


FIG. 2.3 Major mid-ocean rift system (thin continuous lines) and ocean trenches (heavy continuous lines). A, Philippine Trench; B, Marianas Trench; C, Vityaz Trench; D, New Hebrides Trench; E, Peru–Chile Trench; F, Puerto Rico Trench. The East Pacific Ridge is also known as the East Pacific Rise.

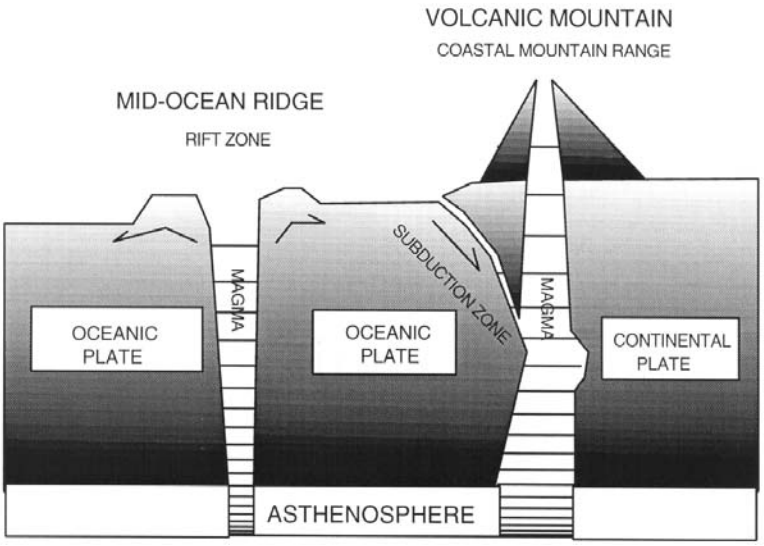


FIG. 2.4 Schematic representation of seafloor spreading and plate subduction. New oceanic crust is formed at the rift zone of the mid-ocean ridge. Old oceanic crust is consumed in the subduction zone near a continental margin or island arc.

adjacent parts of the plates to be pushed away from the ridge in opposite directions (Fig. 2.4). The oldest portions of oceanic plates are consumed by subduction more or less in proportion to the formation of new oceanic plate at the mid-ocean ridges, thereby maintaining a fairly constant plate size.

Volcanism occurs not only at mid-ocean ridges but also in the regions of subduction where the sinking crustal rock undergoes melting as it descends toward the upper mantle. The molten rock may then erupt through fissures in the crust and contribute to mountain building at the continental margins (**orogeny**). It is plate collision and volcanic activity associated with subduction at continental margins that accounts mainly for the existence of coastal mountain ranges. The origin of the Rocky Mountains and the Andes on the North and South American continents, respectively, is associated with subduction activity, whereas the Himalayas are the result of collision of the plate holding the Indian subcontinent with that holding the Asian continent.

Volcanic activity may also occur away from crustal plate margins, at so-called *hot spots*. In the Pacific Ocean, one such hot spot is represented by the island of Hawaii with its active volcanoes. The remainder of the Hawaiian island chain had its origin at the same hot spot where the island of Hawaii is presently located. Crustal movement of the Pacific Ocean plate westward caused

the remaining islands to be moved away from the hot spot so that they are no longer volcanically active.

The continents as they exist today are thought to have derived from a single continental mass, **Pangaea**, which broke apart due to crustal movement less than 200 million years ago. Initially this separation gave rise to **Laurasia** (which included present-day North America, Europe, and most of Asia) and **Gondwana** (which included present-day Africa, South America, Australia, Antarctica, and the Indian subcontinent). These continents separated subsequently into the continents we know today, except for the Indian subcontinent, which did not join the Asian continent until some time after this breakup (Fig. 2.5) (Dietz and Holden, 1970; Fooden, 1972; Matthews, 1973; Palmer, 1974; Hoffman, 1991; Smith, 1992). The continents that evolved became modified by accretion of small landmasses through collision with plates bearing them. Pangaea itself is thought to have originated 250–260 million years ago from an aggregation of crustal plates bearing continental landmasses including Baltica (consisting of Russia west of the Ural Mountains, Scandinavia, Poland, and Northern Germany), China, Gondwana, Kazakhstania (consisting of present-day Kazakhstan), Laurentia (consisting of most of North America, Greenland, Scotland, and the Chukotski Peninsula of eastern Russia), and Siberia (Bambach et al., 1980). Mobile continental plates are believed to have existed as long as 3.5 billion years ago (Kroener and Layer, 1992).

The evidence for the origin and movement of the present-day continents rests on at least three kinds of studies: paleomagnetic and seismic examinations of the Earth's crust, comparative sedimentary analyses of deep-ocean cores obtained from drillings by the *Glomar Challenger*, an ocean-going research vessel, and paleoclimatic studies (Bambach et al., 1980; Nierenberg, 1978; Vine, 1970; Ritter, 1999). Although the separation of the present-day continents had probably no significant effect on the evolution of prokaryotes (they had pretty much evolved to their present complexity by this time), it did have a profound effect on the evolution of metaphytes and metazoans (McKenna, 1972; Raven and Axelrod, 1972). Flowering plants, birds, and mammals, for example, had yet to establish themselves.

2.2 THE BIOSPHERE

The **biosphere**, that portion of the planet that supports life, is restricted to the uppermost part of the crust and, to a degree, to the lowermost part of the atmosphere. It includes the land surface, i.e., the exposed sediment or soil and rock to a limited depth, sometimes called the *lithosphere* by ecologists (see Sec. 2.1 for geologists' definition), and the hydrosphere, that portion of the crust covered by water. Although on land most life exists at the surface, significant

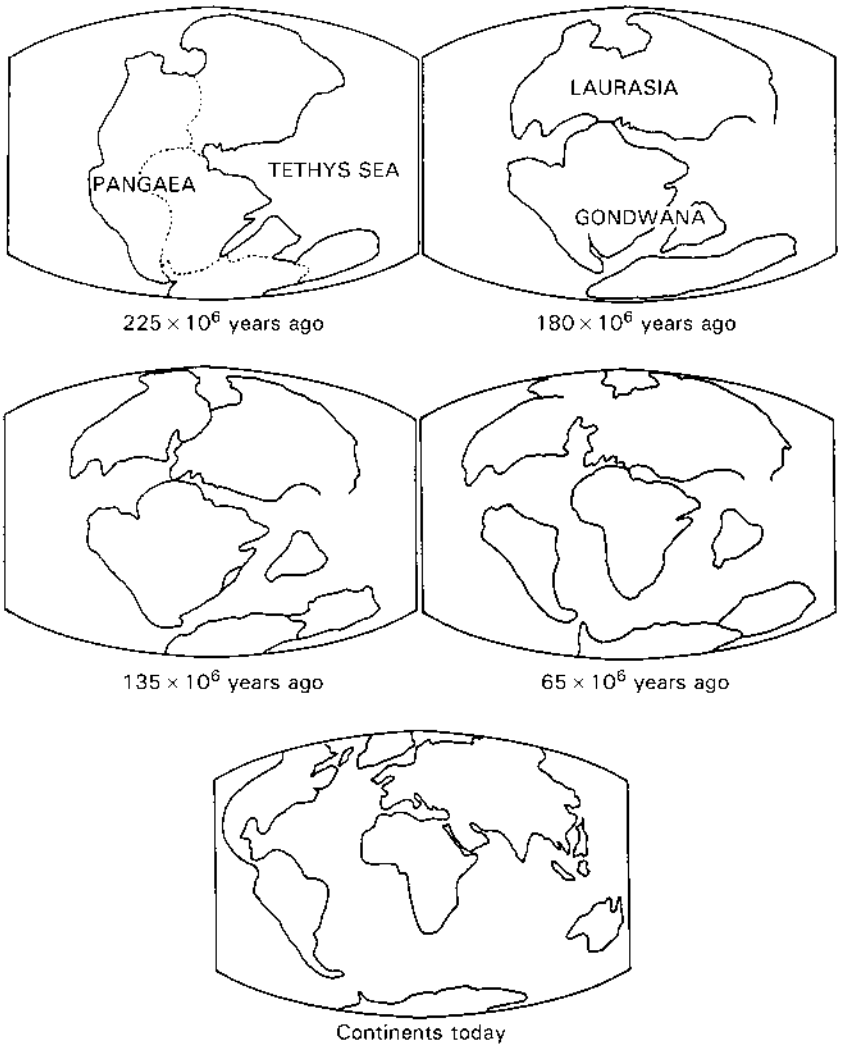


FIG. 2.5 Continental drift. Simplified representation of the breakup of Pangaea to present time. (Reproduced from Dietz and Holden, 1970.)

populations of microbes have now been detected in various sedimentary rock strata at depths of hundreds of meters and more (Ghiorse and Wilson, 1988; Pedersen, 1993). Life in the exposed crust or lithosphere on land was claimed by Pokrovskiy (cited by Kuznetsov et al., 1963, p. 26) to exist to a depth as great as

4000 m. Much more recently, a controlled study confirmed the presence of life in groundwater from a depth of 3500 m from a borehole in granitic rock in the Siljan Ring in central Sweden (Szewzyk et al., 1994). The water from this depth contained thermophilic, anaerobic fermenting bacteria related to *Thermoanaerobacter* and *Thermoanaerobium* species and one strain related to *Clostridium thermohydrosulfuricum* but no sulfate-reducing or methanogenic bacteria. The bacteria that were cultured grew in a temperature range of 45–75°C (65°C optimum) at atmospheric pressure in the laboratory. In continental crust, the temperature has been estimated to increase by about 25°C per kilometer of depth (Fredrickson and Onstott, 1996). Using this constant, the in situ temperature at a depth of 3500 m should be about 87.5°C, which is higher than the maximum temperature tolerated by the cultures isolated by Szewzyk et al. (1994) when grown under laboratory conditions, but well within the temperature range of hyperthermophilic bacteria (present maximum growth temperature about 110°C). Within a very limited range, elevated hydrostatic pressure to which microbes would be subjected at greater depths may increase their temperature tolerance slightly, as suggested by the observations of Haight and Morita (1962) and Morita and Haight (1962). Clearly temperature and hydrostatic pressure are important determinants of the depth limit at which life can exist within the crust. Other important limiting factors are porosity and the availability of nutrients and moisture (Colwell et al., 1997).

Unlike the lithosphere, the hydrosphere is inhabited by life at all water depths, some as great as 11,000 m, the depth of the Marianas Trench. In marine sediments, microbial life has now been detected at depths of >500 mbsf (meters below the surface) (Parkes et al., 1994; Cragg et al., 1996). Bacterial alteration of the glass in ocean basalts has been seen to decreasing extents for 250–500 mbsf (Torsvik et al., 1998; Furnes and Staudigel, 1999). In some parts of the hydrosphere, some special *ecosystems* have evolved whose primary energy source is geothermal rather than radiant energy from the sun (Jannasch, 1983). These ecosystems occur around hydrothermal vents at mid-ocean rift zones. Here heat from magma chambers diffuses upward into overlying basalt, causing seawater that has penetrated deep into the basalt to react with it (see Chap. 16, Fig. 16.17, for a diagrammatic representation of this process). This seawater–basalt interaction results in the formation of hydrogen sulfide and in the solution of some metals, particularly iron and manganese and in some cases some other metals such as copper and zinc. The altered seawater (now a **hydrothermal solution**) charged with these solutes is eventually forced up through cracks and fissures in the basalt to enter the overlying ocean through hydrothermal vents. Autotrophic bacteria living free around the vents or in symbiotic association with some metazoa at these sites use the hydrogen sulfide as an energy source for converting carbon dioxide into organic matter. Some of this organic matter is used as food by heterotrophic microorganisms and metazoa at these locations (Jannasch, 1983;

Tunnicliffe, 1992). The hydrogen sulfide–oxidizing bacteria are the chief **primary producers** in the ecosystems, taking the place of photosynthesizers such as cyanobacteria, algae, or plants, the usual primary producers on Earth. Photosynthesizers cannot operate in the location of hydrothermal vent communities because of the perpetual darkness that prevails at these sites (see also Chap. 18, Sec. 18.8).

Not all submarine communities featuring chemosynthetic hydrogen sulfide oxidizers as primary producers are based on hydrothermal discharge. On the Florida Escarpment in the Gulf of Mexico, ventlike biological communities have been found at abyssal depths around hydrogen sulfide seeps whose discharge is at ambient temperature. The sulfide in this instance may originate from an adjacent carbonate platform containing fluids with 250‰ dissolved solids and temperatures up to 115°C (Paul et al., 1984).

In some other instances, such as the Oregon Subduction Zone or at some sites on the Florida Escarpment, methane of undetermined origin expelled from the pore fluids of the sediments, rather than hydrogen sulfide, is the basis for primary production on the seafloor. Metazoa share in the carbon fixed by free-living or symbiotic methane-oxidizing bacteria (Kulm et al., 1986; Childress et al., 1986; Cavanaugh et al., 1987) (see also Chap. 21).

Finally, the biosphere includes the lower portion of the atmosphere. Living microbes have been recovered from it at heights as great as 48–77 km above the Earth's surface (Imshenetsky et al., 1978; Lysenko, 1979).

Whether the atmosphere constitutes a true microbial habitat is very debatable. Although it harbors viable vegetative cells and spores, it is generally not capable of sustaining growth and multiplication of these organisms because of lack of sufficient moisture and nutrients and because of lethal radiation, especially at higher elevations. At high humidity in the physiological temperature range, some bacteria may, however, propagate to a limited extent (Dimmick et al., 1979; Straat et al., 1977). The residence time of microbes in air may also be limited, owing to eventual fallout. In the case of microbes associated with solid particles suspended in still air, the fallout rate may range from 10^{-3} cm sec^{-1} for particles in a 0.5 μm size range to 2 cm sec^{-1} for particles in a 10 μm size range (Brock, 1974, p. 541). Even if it is not a true habitat, the atmosphere is nevertheless important to microbes. It is a vehicle for spreading microbes from one site to another; it is a source of oxygen for strict and facultative aerobes; it is a source of nitrogen for nitrogen-fixing microbes; and its ozone layer screens out most of the harmful ultraviolet radiation from the sun.

Although the biosphere is restricted to the uppermost crust and the atmosphere, the core of the Earth does exert an influence on some forms of life. The core, with its solid center and molten outer portion, acts like a dynamo in generating the magnetic field surrounding the Earth (Strahler, 1976, p. 36; Gubbins and Bloxham, 1987; Su et al., 1996; Glatzmaier and Roberts, 1996).

Magnetotactic bacteria, because they form magnetite or iron sulfide crystals in their cells that behave like compasses, can utilize the Earth's magnetic field for purposes of orientation in seeking their preferred habitat, which is a partially reduced environment. They are able to align themselves with respect to the Earth's magnetic field (Blakemore, 1982; DeLong et al., 1993).

2.3 SUMMARY

The surface of the Earth includes the lithosphere, hydrosphere, and atmosphere, all of which are habitable by microbes to a greater or lesser extent and constitute the biosphere of the Earth.

The structure of the Earth can be separated into the core, the mantle, and the crust. Of these, only the uppermost portion of the crust is habitable by living organisms. The crust is not a continuous solid layer over the mantle, but consists of a number of crustal plates afloat on the mantle, or more specifically on the asthenosphere of the mantle. Some of the plates lie entirely under the oceans. Others carry parts of a continent or parts of a continent and an ocean. Oceanic plates are growing along mid-ocean spreading centers, while old portions are being destroyed by subduction under or collision with continental plates. The crustal plates are in constant, albeit slow, motion owing to the action of convection cells in the underlying mantle. The plate motion accounts for continental drift.

REFERENCES

Anderson DL. 1992. The earth's interior. In: Brown CG, Hawksworth CJ, Wilson RCL, eds. Understanding the Earth. Cambridge, UK: Cambridge Univ Press, pp 44–66.

Bambach RK, Scotese CR, Ziegler AF. 1980. Before Pangaea: The geography of the Paleozoic world. Am Sci 68:26–38.

Blakemore RP. 1982. Magnetotactic bacteria. Annu Rev Microbiol 36:217–238.

Bonnatti E, Hamlyn PR. 1978. Mantle uplifted block in western Indian Ocean. Science 201:249–251.

Brock TD. 1974. Biology of Microorganisms. 2nd ed. Englewood Cliffs, NJ: Prentice-Hall.

Cavanaugh CM, Levering PR, Maki JS, Mitchell R, Lidstrom ME. 1987. Symbiosis of methylotrophic bacteria and deep-sea mussels. Nature (Lond) 325:346–348.

Childress JJ, Fischer CR, Brooks JM, Kennecutt MC II, Bidigare R, Anderson AE. 1986. A methanotrophic marine molluscan (Bivalvia, Mytilidae) symbiosis: Mussels fueled by gas. Science 233:1306–1308.

Colwell FS, Onstott TC, Delwiche ME, Chandler D, Fredrickson JK, Yao Q-J, McKinley JP, Boone DR, Griffiths R, Phelps TJ, Ringelberg D, White DC, LaFreniere L, Balkwill D, Lehman RM, Konisky J, Long PE. 1997. Microorganisms from deep, high temperature sandstones: Constraints on microbial colonization. FEMS Microbiol Rev 20:425–435.

Cragg BA, Parkes RJ, Fry JC, Weightman AJ, Rochelle PA, Maxwell JR. 1996. Bacterial populations and processes in sediments containing gas hydrates (OPD Leg 146: Cascadia Margin). Earth Planet Sci Lett 139:497–507.

DeLong EF, Frankel RB, Bazylinski DA. 1993. Multiple evolutionary origin of magnetotaxis. Science 259:803–806.

Dietz RC, Holden JC. 1970. Reconstruction of Pangaea: Breakup and dispersion of continents, Permian to present. J Geophys Res 75:4939–4956.

Dimmick RL, Wolochow H, Chatigny MA. 1979. Evidence that bacteria can form new cells in air-borne particles. Appl Environ Microbiol 37:924–927.

Fooden J. 1972. Breakup of Pangaea and isolation of relict mammals in Australia, South America, and Madagascar. Science 175:894–898.

Fredrickson JK, Onstott TC. 1996. Microbes deep inside the Earth. Sci Am 275: 68–83.

Furnes H, Staudigel H. 1999. Biological mediation in ocean crust alteration: How deep is the deep biosphere? Earth Planet Sci Lett 166:97–103.

Ghiorse WC, Wilson JT. 1988. Microbial ecology of the terrestrial subsurface. Adv Appl Microbiol 33:107–171.

Glatzmaier GA, Roberts PH. 1996. Rotation and magnetism of Earth's inner core. Science 274:1887–1891.

Gubbins D, Bloxham J. 1987. Morphology of the geomagnetic field and implications for the geodynamo. Nature (Lond) 325:509–511.

Gurnis M. 1992. Rapid continental subsidence following initiation and evolution of subduction. Science 255:1556–1558.

Haight RD, Morita RY. 1962. Interaction between the parameters of hydrostatic pressure and temperature on aspartase of *Escherichia coli*. J Bacteriol 83:112–120.

Hoffman PF. 1991. Did the breakup of Laurentia turn Gondwanaland inside-out? Science 252:1409–1412.

Imshenetsky AA, Lysenko SV, Kazatov GA. 1978. Upper boundary of the biosphere. Appl Environ Microbiol 35:1–5.

Jannasch HW. 1983. Microbial processes at deep sea hydrothermal vents. In: Rona PA, Bostrom K, Laubier L, Smith KL Jr, eds. Hydrothermal Processes at Sea Floor Spreading Centers. New York: Plenum, pp 677–710.

Kay M. 1955. Sediments and subsidence through time. In: Poldervaart A, ed. Crust of the Earth: A Symposium. Spec Pap 62A. New York: Geological Society of America, pp 665–684.

Kearey P, ed. 1993. The Encyclopedia of the Solid Earth Sciences. Oxford, UK: Blackwell, p 472.

Kerr RA. 1995. Earth's surface may move itself. Science 269:1214–1215.

Kroener A, Layer PW. 1992. Crust formation and plate motion in the Early Archean. Science 256:1405–1411.

Kulm LD, Suess E, Moore JC, Carson B, Lewis BT, Ritger SD, Kadko DC, Thornburg TM, Ebley RW, Rugh WD, Maasoth GJ, Lagseth MG, Cochrane GR, Scamman RL. 1986. Oregon subduction zone: Venting, fauna, and carbonates. Science 231:561–566.

Kuznetsov SI, Ivanov MV, Lyalikova NN. 1963. Introduction to Geological Microbiology. English transl. New York: McGraw-Hill.

Lysenko SV. 1979. Microorganisms in the upper atmospheric layers. Mikrobiologiya 48:1066–1074 (Engl transl 871–877).
Madon M. 1992. Mantle. In: Nierenberg WA, ed. Encyclopedia of Earth System Science, Vol 3. San Diego, CA: Academic Press, pp 85–99.
Matthews SW. 1973. This changing Earth. Natl Geogr 143:1–37.
McKenna MC. 1972. Possible biological consequences of plate tectonics. BioScience 22:519–525.
Mercy E. 1972. Mantle geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 677–683.
Morita RY, Haight RD. 1962. Malic dehydrogenase activity at 101°C under hydrostatic pressure. J Bacteriol 83:1341–1346.
National Geographic Society. 1995. The Earth's fractured surface (map supplement). Natl Geogr 187(4).
Nierenberg WA. 1978. The deep sea drilling project after ten years. Am Sci 66:20–29.
Palmer AR. 1974. Search for the Cambrian world. Am Sci 62:216–224.
Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightman AJ, Harvey SM. 1994. Deep bacterial biosphere in Pacific Ocean sediments. Nature (Lond) 371:410–413.
Paul CK, Hecker B, Commeau R, Freeman-Lynde RP, Neumann C, Corso WP, Golubic S, Hook JE, Sikes E, Curray J. 1984. Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. Science 226:965–967.
Pedersen K. 1993. The deep subterranean biosphere. Earth-Sci Rev 34:243–260.
Raven PH, Axelrod DI. 1972. Plate tectonics and Australian paleogeography. Science 176:1379–1386.
Ritter JRR. 1999. Rising through Earth's mantle. Science 286:1865–1866.
Ronov AB, Yaroshevsky AA. 1972. Earth's crust and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 243–254.
Skinner BJ, Porter SC, Botkin DB. 1999. The Blue Planet. An Introduction to Earth System Science. 2nd ed. New York: Wiley.
Smith AG. 1992. Plate tectonics and continental drift. In: Brown G, Hawkesworth C, Wilson C, eds. Understanding the Earth. Cambridge, UK: Cambridge Univ Press, pp 187–203.
Straat PA, Woodrow H, Dimmick RL, Chatigny MA. 1977. Evidence for incorporation of thymidine into deoxyribonucleic acid in air-borne bacterial cells. Appl Environ Microbiol 34:292–296.
Strahler N. 1976. Principles of Physical Geology. New York: Harper and Row.
Su W-j, Dziewonski AM, Jeanloz R. 1996. Planet within a planet: Rotation of the inner core of Earth. Science 274:1883–1887.
Szewzyk U, Szewzyk R, Stenström T-A. 1994. Thermophilic, anaerobic bacteria isolated from a deep borehole in granite in Sweden. Proc Natl Acad Sci USA 91:1810–1813.
Torsvik T, Furness H, Muehlenbachs K, Thorseth IH, Tumyr O. 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. Earth Planet Sci Lett 162:165–176.

Tunnicliffe V. 1992. Hydrothermal-vent communities. Am Sci 80:336–349.
Van Andel TH. 1992. Seafloor spreading and plate tectonics. In: Brown G, Hawkesworth C, Wilson C, eds. Understanding the Earth. Cambridge, UK: Cambridge Univ Press, pp 167–186.
Vine FJ. 1970. Spreading of the ocean floor: New evidence. Science 154:1405–1415.
Wood BJ. 1997. Hydrogen: An important constituent of the core? Science 278:1727.
Wysession M. 1995. The inner workings of the Earth. Am Sci 83:134–147.

3

The Origin of Life and Its Early History

3.1 THE BEGINNINGS

The Earth is thought to be about 4.6×10^9 years old (4.6 eons). One accepted view holds that it derived from an accretion disc that resulted from gravitational collapse of interstellar matter. A major portion of the matter condensed to form the Sun, a star. Other components in the disc subsequently accreted to form planetesimals of various sizes. These in turn accreted to form our Earth and the other inner planets of our solar system, namely, Mercury, Venus, and Mars. All these inner planets are rocky. As accretion of the Earth proceeded, its internal temperature could have risen sufficiently to result ultimately in separation of silicates and iron, leading to a differentiation into mantle and core. Alternatively, and more likely, a primordial rocky core could have been displaced by a liquid iron shell that surrounded it. Displacement of the rocky core would have been made possible if it fragmented as a result of nonhydrostatic pressures, causing the inner iron core to become surrounded by a hot, well-mixed mantle or rock material in a *catastrophic process*. Whichever process actually took place, much heat must have been released during this formational process, resulting in outgassing from the mantle to form a primordial atmosphere and, possibly, hydrosphere. It has been suggested recently that bombardment of the early Earth by giant comets that consisted of water ice and cosmic dust introduced much of the water on the Earth's surface (see, for instance, Delsemme, 2001;

Broad, 1997). All this is thought to have occurred in a span of about 10^8 years. As the planet cooled, segregation of mantle components is thought to have occurred and a thin crust to have formed by 4.0–3.8 eons ago. Accretion by meteorite (bolide) bombardment is believed to have become insignificant by this time. The first crustal plates may have formed between 3.8 and 2.7 eons ago, initiating tectonic activity in the crust that continues to this day. Protocontinents may have emerged at this time to be subsequently followed by the development of true continents. (For details about these early steps in the formation of the Earth, see Stevenson, 1983; Ernst, 1983; Taylor, 1992.) How did life originate on this newly formed Earth?

Origin of Life on Earth—Panspermia

According to the **panspermia** hypothesis, life arrived on this planet preformed in the form of one or more kinds of spores from another world. This view finds some support in laboratory studies published by Weber and Greenberg (1985). These studies employed spores of *Bacillus subtilis*, a common soil bacterium, enveloped in a mantle of 0.5 μm thickness or greater derived from equal parts of H_2O, CH_4, NH_3, and CO (presumed interstellar conditions). The mantle shielded the spores from short ultraviolet (UV) radiation (100–200 μm wavelength) in ultrahigh vacuum ($<1 \times 10^6$ torr) at 10°K but not from long-UV radiation (200–300 μm). From experimentally determined survival rates of the spores, the investigators calculated that if spores were enveloped in a mantle of 0.9 μm thickness having a refractive index of 0.5, which would protect them from short- and long-wavelength UV, they could survive in sufficient numbers over a period of 4.5–45 million years (Myr) in outer space to allow them to travel from one solar system to another. Spores could have entered outer space in high-speed ejecta as a result of collisions between a life-bearing planet and a meteorite or comet (Weber and Greenberg, 1985).

Instead of individual spores coated in a mantle of H_2O, CH_4, NH_3, and CO arriving on the Earth's surface, it is possible that spores were carried inside ejecta of rock fragments generated by a meteorite impact on another planet that harbored life (Cohen, 1995; Nicholson et al., 2000). As shown in other parts of this book (e.g., Chap. 8), microbial life is known to exist inside some rocks on Earth, and thus the idea of viable spores inside ejecta of rock fragments is not preposterous. If such rock fragments are large enough, shock-induced heating and pressure through meteorite impact and the acceleration that an ejected rock fragment would undergo immediately after meteorite impact could be survived by bacterial spores inside the rock fragment (for more details see Nicholson et al., 2000). Enclosure in a protective film such as ice consisting of H_2O, CH_4, NH_3, and CO in a polysaccharide film or in a salt crystal is thought to enable spores to survive the dehydrating effect of high vacuum of space (see Weber and

Greenberg, 1985; Nicholson et al., 2000). Enclosure in a rock fragment is thought to protect spores sufficiently not only from UV but also from cosmic ionizing radiation to survive interplanetary travel (Nicholson et al., 2000). Furthermore, spores in a large enough rock fragment should be able to survive entry into and penetration of Earth's atmosphere and subsequent impact on the Earth. Breakup of the entering rock fragment due to aerodynamic drag in the lower atmosphere would ensure scattering of the inoculum at the Earth's surface (see Nicholson et al., 2000 for more detail).

Despite the possibility that life on Earth could have originated elsewhere in the universe, a more widely held view is that life began de novo on Earth.

Origin of Life on Earth—De Novo Appearance

For life to have originated de novo on Earth, the existence of a primordial nonoxidizing atmosphere was of primary importance. There is no common agreement as to whether the atmosphere was reducing or nonreducing. Constituents may have included H_2O, H_2, CO_2, CO, CH_4, N_2, NH_3 (see Table 4.3 in Chang et al., 1983), and HCN (Chang et al., 1983). The exact composition of the atmosphere will have changed as time progressed. The cause of this change will have been photochemical reactions and reactions driven by electric discharge (lightning) in the atmosphere, interaction of some of the gases with mineral constituents at high temperature, and escape of the lightest gases (e.g., hydrogen) into space (Chang et al., 1983; Schopf et al., 1983). Two opposing views are currently held on how life may have arisen de novo on Earth.

Life from Abiotically Formed Organic Molecules in Aqueous Solution ("Organic Soup Theory")

An older view, and one that is still favored, is that life arose in a dilute "organic soup (broth)" that covered the surface of the planet. This view developed out of the proposals of Haldane (1929) and Oparin (1938). According to it, the biologically important organic molecules in the soup were formed by abiotic chemical interactions among some of the atmospheric gases, driven by heat, electric discharge, and/or light energy (see discussion by Chang et al., 1983). If, as Bada et al. (1994) have theorized, the surface of the early Earth was frozen because the sun was less luminous than it was to become later, bolide impacts could have caused episodic melting, during which time the abiotic reactions took place. Alternatively it is possible that few or none of the early organic molecules in the organic soup were formed on Earth, with most or all of them being introduced on the Earth's surface by collision with giant comets. Whatever the origin of these molecules, special polymeric molecules that had the ability to self-reproduce (the beginning of true life) arose abiotically at the expense of certain

organic molecules (building blocks) that continued to be abiotically synthesized and/or were introduced on Earth by comet bombardment. Clays could have played an important role as catalysts and templates in the assembly of the polymeric molecules (Cairns-Smith and Hartman, 1986). Ribonucleic acid (RNA) may have been the most important original polymeric molecule (Gilbert, 1986; Joyce, 1991) that was able to self-assemble autocatalytically from abiotically formed nucleotides, according to the findings by Cech (1986), Doudna and Szostak (1989), and others. As this self-reproducing RNA evolved, it acquired new functions through mutations and recombinations, with the result that an RNA world emerged. In time, a form of RNA (template RNA) arose that assumed a direct role in the assembly of proteins from constituent amino acids. Many of these proteins were enzymes (biocatalysts), and among these proteins were some that assumed a catalytic role in RNA synthesis. The protein catalysts were more efficient than RNA catalysts (Gilbert, 1986). Still later, deoxyribonucleic acid (DNA), which may have arisen independently of RNA, acquired information stored in RNA by a process of reverse transcription, a process in which information stored in RNA was transcribed into DNA (Gilbert, 1986). This speculative scenario has been proposed as a result of studies in the last two decades in which some RNAs were discovered in living cells that can modify themselves by self-splicing through catalysis of phosphoester cleavage and phosphoester transfer reactions (*ribozyme* activity) (Kruger et al., 1982; Guerrier-Takada et al., 1983; Cech, 1986; Doudna and Szostak, 1989).

The ability of certain RNAs to transform themselves catalytically is not unique to them. Some proteins are also known to catalyze their own transformation. Thus in considering the origin of life on Earth, it cannot be ruled out that proteins with self-reproducing properties arose spontaneously from abiotically formed amino acids (Doebler, 2000). Among these proteins may have been some that were able to catalyze polymerization of abiotically formed building blocks of RNA, the ribonucleotides, into RNAs. Some of these RNAs may subsequently have developed an ability to serve as templates in protein synthesis, making synthesis of specific proteins more orderly. Other RNAs may have evolved into reactants (transfer RNAs) in the protein assembly reactions in which amino acids are linked to each other by peptide bonds. This made the polymerization more efficient. As template RNA became more diverse through mutation and recombination, the diversity of catalytic proteins increased. This resulted in controlled, accelerated synthesis of the building blocks (amino acids, fatty acids, sugars, nucleotides, etc.) from which vital polymers (proteins, lipids, polysaccharides, nucleic acids, etc.) could be synthesized by other newly evolved catalytic proteins. Enzyme-catalyzed synthesis was much more efficient than abiotic synthesis.

We may assume that in order to optimize the various biochemical processes that had become interdependent or had a potential for it, cells became encapsu-

lated. This encapsulation involved enclosure in a lipid membrane, which provided an environment in which vital syntheses could proceed at optimal rates. Whether the first membranes were like the bilipid membranes of cells of today remains unknown. A model for a primitive form of encapsulated cell may be a present-day observation of enzyme-catalyzed RNA synthesis from nucleotides in artificially formed lipid vesicles containing a template-independent polymerase protein. Adenosine diphosphate substrate was found to penetrate such vesicles readily from the exterior solution. It was then attached to some pre-existent RNA in the vesicles by template-independent RNA polymerase (Chakrabarti et al., 1994).

As the primitive cells evolved, special proteins (transport proteins) became introduced into their membranes. These proteins exerted positive or negative control over the entry and exit of specific substances into and out of the cell. In time, the membrane of some cells also acquired an energy-transducing system, the electron transport or respiratory chain, which made metabolic energy conservation more efficient.

According to the organic soup scenario, the first primitive cells that arose in the evolution of life were heterotrophs, which depended on abiotically formed organic building blocks in the organic soup. As time went on, the supply of abiotically formed organic molecules must have become progressively more limiting. This happened because the Earth's surface underwent changes. Conditions for abiotic synthesis became less and less favorable, but the demand for building blocks increased exponentially. The emergence of autotrophs, which had acquired the ability to form their own organic building blocks from inorganic constituents in their surrounding environment by using chemical or radiant energy as the driving force of these reactions, made the heterotrophs independent of the supply of abiotically formed building blocks. They were now able to feed on secretions of excess organic synthate formed by the autotrophs or on their dead remains.

Surface Metabolism Theory

According to one view of how life originated on Earth, life arose as a form of surface metabolism, an autocatalytic process that does not involve enzymes or templates. This theory was first proposed by Wächtershäuser (1988). According to it, building blocks were synthesized and polymerized starting with inorganic constituents (carbon dioxide, phosphate, ammonia) on the surface of minerals with a positive (anodic) surface charge (Wächtershäuser favors iron pyrite, FeS_2). It is a given for Wächtershäuser (1988) that polymerizations of surface-bound molecules are thermodynamically favorable, whereas polymerizations of the same molecules in solution (organic soup scenarios) are thermodynamically unfavorable. In the latter case, the water in which the monomers and polymers are dissolved favors hydrolytic cleavage of the polymers. In Wächtershäuser's surface

metabolism scenario, some of the surface reactions are autocatalytic and, because of their ability to replicate the reaction product, constituted the first life forms, which were two-dimensional ("surface metabolists" in Wächtershäuser's terminology). With the emergence of isoprenoid lipid synthesis, surface metabolists eventually became covered by lipid membranes (half-cells) and ultimately were completely enclosed by them. This membrane encapsulation thus produced the first cells. They featured a membrane-enclosed cytosol in which initially the vital chemistry still occurred on the surface of a mineral grain. However, with the passage of time and the appearance of some critical molecules, the vital chemistry became progressively independent of the mineral surface and assumed a distinct existence in the aqueous phase of the cytosol. In the original cells, the mineral grain may have consisted of pyrite (FeS_2), which may have been the product of early energy and reducing-power generation according to Wächtershäuser (1988) (see also below). The appearance of deoxyribonucleic acid and ribonucleic acid, which eventually became the key components of the genetic apparatus and assumed firm control of metabolic behavior and its perpetuation, began independently as a part of surface metabolism in the precellular stage without exerting control over it. The evolution of the genetic apparatus once the cellular stage had been attained involved, among other things, the enscription in DNA via RNA of structural information of specific proteins and the development of a mechanism for deciphering this information in the synthesis of the proteins, many of which are enzymes. After cellular metabolism became independent of the mineral grain, it was controlled by these enzymes.

The appearance of enzymes in the cytosol made possible for the first time the utilization of accumulated, surface-detached organic substances in the cytosol (called salvaging action by Wächtershäuser, 1988). Surface metabolists had been completely unable to use these products.

In contrast to the organic soup theory, the surface metabolism theory proposes that the first life in the attached and detached states was **autotrophic**, i.e., it depended on CO_2, CO, NH_3, H_2S, H_2, and H_2O to form organic molecules. Driving energy and reducing power for autotrophic metabolism may have come from an interaction of ferrous iron and hydrogen sulfide (Wächtershäuser, 1988):

$$Fe^{2+} + H_2S \rightarrow FeS_2 + 2H^+ + 2e \qquad (\Delta G^\circ,\ -2.62 \text{ kcal or } -11 \text{ kJ}) \tag{3.1}$$

Both the Fe(II) and H_2S should have been plentiful on the primitive Earth. The formation of pyrite and H_2 from a reaction between FeS and H_2S under fastidiously anaerobic conditions was experimentally demonstrated by Drobner et al. (1990):

$$FeS + H_2S \rightarrow FeS_2 + H_2 \qquad (\Delta G^\circ,\ -6.14 \text{ kcal or } -25.7 \text{ kJ}) \tag{3.2}$$

The H_2 then served as energy source and reductant in further metabolism.

Evolution of a membrane-bound respiratory chain can be assumed to have followed the formation of the cell membrane. According to Wächtershäuser (1988), the respiratory chain liberated organisms that developed it from having to use reactions of ferrous iron with H_2S as a source of energy and reducing power by enabling them to use reactions like those in which H_2 reduces elemental sulfur or sulfate to H_2S (see Chap. 18). Autotrophic catabolism is also seen as the basis for the emergence of substrate-level phosphorylation (see Chap. 6) as an alternative means of conserving energy. *Heterotrophy*, in which energy needed by the cell is generated in the oxidation of reduced carbon compounds, is believed by Wächtershäuser (1988) to have evolved from autotrophic catabolism in some cell lines. This required the development of a transport mechanism across the cell membrane for importing dissolved organic molecules from the environment surrounding the cells. He believes that this occurred at a late stage in the evolution of cells. The first prokaryotic anoxic photosynthesizers probably appeared some time before the first heterotrophs appeared. Thus in the surface metabolism scenario, autotrophy preceded heterotrophy, and anaerobic chemosynthetic autotrophy preceded anoxygenic photosynthetic autotrophy.

Origin of Life Through Iron Monosulfide Bubbles in the Hadean Ocean at the Interface of Sulfide-Bearing Hydrothermal Solution and Iron-Bearing Ocean Water

In a newer proposal by Russell and Hall (1997), bubbles coated by iron monosulfide are postulated to form on the surface of sulfide mounds resulting from hydrothermal seepage on the ocean floor. They are thought to have formed at the solution interface that existed between hot (~150°C), extremely reduced, alkaline, bisulfide-bearing solution and warm (~90°C), iron-bearing, acidic, Hadean ocean water about 4.2 eons ago. The iron monosulfide coating of the bubbles included some nickel and acted like a semipermeable membrane. Its catalytic property depended on the redox potential and pH gradient (acid outside) across it with a ΔE of about 300 mV. Organic anions, formed by the membrane from reactants in the seawater, accumulated inside the bubbles and generated osmotic pressure that kept the bubbles from collapsing. Indeed, continuing solute accumulation inside a bubble could have led to its budding and splitting in two. The simple organic molecules inside the bubbles eventually polymerized with the help of pyrophosphate hydrolysis. The pyrophosphate resulted from a primitive form of chemiosmosis (see Chap. 6). Eventually, the iron monosulfide membrane was replaced by an organic (phospholipid) membrane.

3.2 EVOLUTION OF LIFE THROUGH THE PRECAMBRIAN: BIOLOGICAL AND BIOCHEMICAL BENCHMARKS

The Precambrian [4.5–0.54 eons before the present (b.p.)] may be divided into three eras: the Hadean (4.5–3.8 eons b.p.), the Archean (4.5–2.5 eons b.p.), and the Proterozoic (2.5–0.54 eons b.p.). The Archean may be subdivided into the Early Archean (3.8–3.3 eons b.p.), the Middle Archean (3.3–2.9 eons b.p.), and the Late Archean (2.9–2.5 eons b.p.). The Proterozoic may be subdivided into Early Proterozoic (2.5–1.6 eons b.p.), Middle Proterozoic (1.6–0.9 eons b.p.), and Late Proterozoic (0.9–0.54 eons b.p.) (Table 3.1). The Hadean era was a time before solid rock appeared on the Earth's surface.

Current thinking based on some fossil finds and recent geochemical evidence is that the first life, i.e., the first living entities, whatever their form,

TABLE 3.1 Geologic Time Scale

				Years before the present
Eon:	Precambrian			
	Era:	Archean		$4.5–2.5\times10^9$
		Period:	Hadean	$4.5–3.9\times10^9$
			Early Archean	$3.9–2.9\times10^9$
			Late Archean	$2.9–2.5\times10^9$
	Era:	Proterozoic		$2.5–0.57\times10^9$
		Period:	Early Proterozoic	$2.5–1.6\times10^9$
			Middle Proterozoic	$1.6–0.9\times10^9$
			Late Proterozoic	$0.9–0.57\times10^9$
Eon:	Phanerozoic			
	Era:	Paleozoic		$570–225\times10^6$
		Period:	Cambrian	$570–500\times10^6$
			Ordovician	$500–430\times10^6$
			Silurian	$430–395\times10^6$
			Devonian	$395–345\times10^6$
			Carboniferous	$345–280\times10^6$
			Permian	$280–225\times10^6$
	Era:	Mesozoic		$225–65\times10^6$
		Period:	Triassic	$225–190\times10^6$
			Jurassic	$190–136\times10^6$
			Cretaceous	$136–65\times10^6$
	Era:	Cenozoic		
		Period:	Tertiary	$65–1\times10^6$
			Quaternary	1×10^6–present

could have originated as early as 3.8 billion years ago or earlier (e.g., 4 billion years ago) (Schopf et al., 1983; Mojzsis et al., 1996; Holland, 1997). One fossilized form of "well-developed" cellular life, which had the form of a **stromatolite** (in this instance a fossilized mat of filamentous microorganisms), was found in chert of the Warrawoona Group in the Pilbara Block of Western Australia. Its age has been determined to be ~3.5 billion years (Fig. 3.1) (Lowe, 1980; Walter et al., 1980). Slightly younger microfossils (3.3–3.5 billion years old) having a recognizable cell type that resembles cyanobacteria have been found in the Early Archean Apex Basalt and Towers Formation of the Warrawoona Group (Fig. 3.2) (Schopf and Packer, 1987). Some other stromatolites of approximately similar age have been reported from limestone in the Fort Victoria greenstone belt of the Rhodesian Archean Craton within Zimbabwe (Orpen and Wilson, 1981).

The discovery of fossils of once-living organisms as old as 3.5 billion years leads to the conclusion that noncellular life and single-celled life must have preceded the emergence of stromatolites by a span of 500 million years. Indeed,

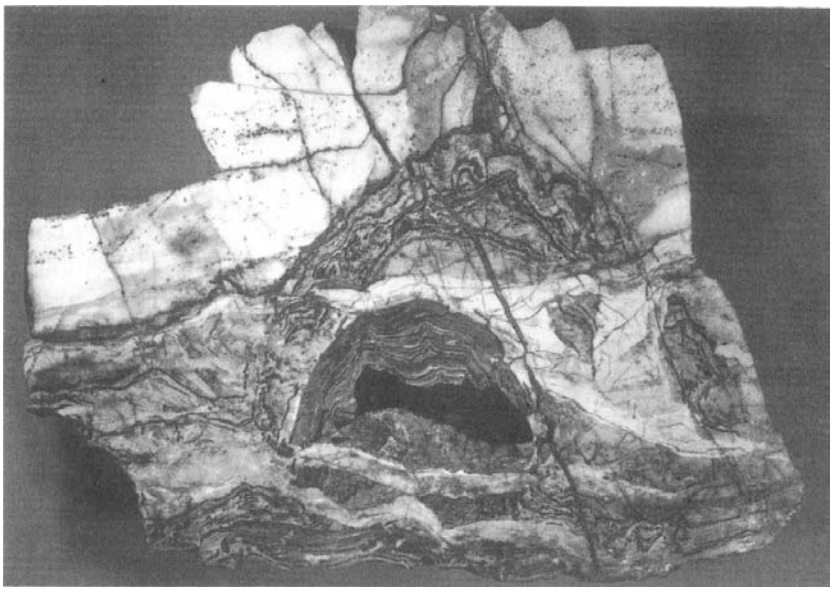


FIG. 3.1 Fossil remnant of ancient life: domical stromatolite (×0.35) from a stratum of the 3.5 billion-year-old Warrawoona Group in the North Pole Dome region of northwestern Australia. A stromatolite is formed from fossilization of a mat of filamentous microorganisms such as cyanobacteria. (Photograph reproduced from the frontispiece of Schopf, 1983, with publisher's permission.)

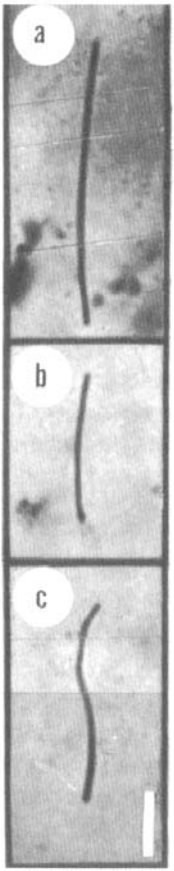


FIG. 3.2 Rod-shaped, threadlike, juvenile forms of apparently nonseptate bacteria in petrographic thin section of stromatolitic black chert from the 3.5 billion-year-old Warrawoona Group (Pilbara Supergroup) of Western Australia. Scale mark in panel c is 5 μm and also applies to panels a and b. (Reproduced from photo 9-4 C, D, and E in Schopf, 1983, with publisher's permission.)

Mojzsis et al. (1996) found carbon isotopic evidence in carbonaceous inclusions (graphitized carbon) within grains of apatite (basic calcium phosphate) from the oldest known sediment sequences that supports the existence of biotic activity. These sediment sequences are the ~3.8 billion-year-old banded iron formation (BIF) of the Isua supracrustal belt of West Greenland and a similar ~3.85 billion-year-old sedimentary formation on the nearby Akilia island. The isotopic signature in this case is represented by a significant enrichment of the graphitized

carbon in the light-stable isotope of carbon, ^{12}C relative to a graphite reference standard (see Chap. 6 for an explanation of isotope enrichment and its significance). The observed magnitude of the enrichment is best explained on a biological basis. An abiotic process is deemed unlikely in this instance (Mojzsis et al., 1996; Holland, 1997). The measurements were made with an ion microprobe.

Early Evolution According to the Organic Soup Scenario

In the organic soup scenario, the precursor molecules such as amino acids, purine and pyrimidine bases, and sugars from which life originated must have appeared in sufficient quantities in the middle to late Hadean. As mentioned in Section 3.1, these monomers must subsequently have polymerized into heteropolymers such as proteins and nucleic acids. The sources of the energy driving the abiotic syntheses of the monomers and their polymerizations were heat, sunlight, and electric discharge.

If we accept the ability to self-reproduce as the basic definition of life, then the appearance of the first proteins and/or nucleic acids with this ability marked the beginning of life. Clays, as already mentioned, may have played an important role in the production of self-reproducing molecules, especially proteins (Cairns-Smith and Hartman, 1986). As discussed in Section 3.1, if proteins were the first living molecules, they would have developed independently of abiotically produced nucleic acids at this stage. If RNAs were the first self-reproducing entities, they may have given rise to templates on which the first proteins were constructed. Regardless of whether the emergence of self-reproducing RNA preceded the emergence of proteins or whether the emergence of self-reproducing proteins preceded the emergence of RNA, lipid vesicles are assumed to have subsequently enclosed these self-reproducing entities to better cope with the environment and make the self-reproduction processes more efficient. These primitive cells must then have evolved systems for intracellular production of the monomers from which the polymers were formed.

If proteins were the first self-reproducing molecules, they and independently evolved ribonucleic acid started to form a replication system in the primitive cells in which the RNA replaced clays as templates in protein synthesis. DNA then evolved to become the repository for structural information of proteins that resided in the RNA templates. Many of these proteins were catalysts needed for the synthesis of monomers and the synthesis of polymers from the monomers. If RNAs were the first living molecules, then their enclosure in primitive cells would have led to the synthesis of proteins through the evolution of template RNA. Many of the proteins would have possessed catalytic functions (Schopf et al., 1983; Miller and Orgel, 1974; Oparin, 1938; Haldane, 1929; Joyce, 1991).

DNA, which evolved subsequently, became a more stable repository for templates and, therefore, protein structure. Whatever their exact sequence, the events probably occurred in the late Hadean.

As the primitive cells evolved, they soon must have developed the traits we associate with modern **prokaryotic cells**, as suggested by micropaleontological evidence. This implies that they possessed a cell envelope or wall surrounding a plasma membrane and enclosing an interior featuring a large deoxyribonucleic acid strand (repository of genetic information), nucleoprotein granules (ribosomes), and other proteins and smaller polymers and monomers. The plasma membrane developed mechanisms for transporting externally available organic and inorganic molecules across it. These first prokaryotes must have been anaerobic **heterotrophs** that could live without free oxygen, because the Earth was surrounded at this time by an atmosphere devoid of oxygen. In the beginning, they may have fed on externally available organic molecules that probably were mostly or entirely abiotically synthesized monomers. However, as the Archean progressed, an evolutionary step was required that would make these cells independent of abiotic syntheses. Such a step was needed because abiotic synthesis was rate-limited so that as cell populations increased exponentially, abiotically synthesized products could no longer meet cellular demand for them. In any case, conditions needed to sustain abiotic synthesis gradually disappeared. To sustain life, **autotrophy** emerged. Autotrophy is a process in which an organism obtains the energy it needs either from the oxidation of an inorganic compound (*chemosynthetic autotrophy* or *chemolithotrophy*) or from the transformation of radiant energy from sunlight into chemical energy (*photosynthetic autotrophy* or *photolithotrophy*). These processes drive the conversion of CO_2 into a form of reduced (organic) carbon. Such autotrophs not only completely satisfied their own needs for reduced carbon monomers from inorganic matter but could also feed the already existing heterotrophs. They did this by excreting reduced carbon monomers that they had synthesized and that were in excess of their needs. Furthermore, after they died, they became food for heterotrophic scavengers.

Whether the first autotrophs were chemosynthetic or photosynthetic is currently a matter of debate. One school of thought favors chemosynthetic autotrophs in the form of **methanogens**, which formed methane according to the reaction

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (\Delta G^\circ = -33.2 \text{ kcal or } -138.8 \text{ kJ}) \qquad (3.3)$$

Such bacteria exist today. They are strict anaerobes. Because a number of the extant methanogens live at relatively high temperatures (+60 to +90°C), not unlike temperatures that must have prevailed on Earth at this time in the Archean (Schopf et al., 1983), and because the large majority of them can use hydrogen, a gas thought to have been sufficiently abundant in the primordial atmosphere, as an energy source, they could have been the first autotrophs to evolve. Analyses by

the techniques of molecular biology have shown that methanogens have a very ancient origin and that they should be grouped in a special domain, the Archaea (initially called Archaebacteria) (Fox et al., 1977; Woese and Fox, 1977). If they were the first autotrophs, they were, however, soon displaced by photosynthetic autotrophs as **primary producers**.

The other school of thought favors photosynthetic prokaryotes in the domain Bacteria as the first autotrophs with equal justification. This notion is supported by the existence of the Warrawoona stromatolite, which is ~3.5 billion years old (Schopf et al., 1983; Schopf and Packer, 1987). This microfossil has been interpreted, on the basis of comparison with modern counterparts, to have been formed by **cyanobacteria** (once called blue-green algae). However, modern cyanobacteria are aerobes, which usually release oxygen when photosynthesizing. Because the primordial atmosphere at this time is thought to have been lacking in oxygen, the emergence of anaerobic photosynthetic bacteria, of which modern purple and green bacteria must be a counterpart, must have preceded that of cyanobacteria. Indeed, recent molecular analysis of photosynthesis genes suggests that purple bacteria represent the oldest photosynthetic prokaryotes and that cyanobacteria were evolutionarily relative latecomers (Xiong et al., 2000; see also Des Marais, 2000). Like their modern counterparts, the anaerobic bacteria photosynthesized without producing oxygen. They transformed radiant energy from sunlight into chemical energy that enabled them to reduce CO_2 to organic carbon with H_2 or H_2S instead of H_2O. Elemental sulfur or sulfate was produced if H_2S was the reductant of CO_2, e.g.,

$$2H_2S + CO_2 \rightarrow (CH_2O) + H_2O + 2S \tag{3.4}$$

where (CH_2O) represents a form of reduced carbon with a ratio of C to H to O of 1:2:1. Such organisms would have kept the Archean atmosphere oxygen-free.

The emergence of anaerobic (anoxygenic) photosynthesis required the appearance of **chlorophyll**, the light-harvesting and energy-transducing pigment, in the process (Chap. 6). A modification of this chlorophyll was required to enable the emergence of the oxygenic phototrophs, i.e., the cyanobacteria-like organisms. This modification enabled them to substitute H_2O for H_2 or H_2S as a reducing agent of CO_2, leading to the introduction of oxygen into the atmosphere:

$$H_2O + CO_2 \rightarrow (CH_2O) + O_2 \tag{3.5}$$

A few cyanobacteria are now known that have the capacity to carry out not only *oxygenic* photosynthesis but also *anoxygenic* photosynthesis under anaerobic conditions when H_2S is present. In the latter instance they photosynthesize like purple or green bacteria, using the H_2S rather than water to reduce CO_2 (Cohen et al., 1975). This means that the microfossils that Schopf and Packer (1987) found in 3.3–3.5 billion-year-old chert from the Warrawoona Group in Western Australia may have been cyanobacteria that were photosynthesizing anoxygenically or were just beginning to develop an oxygenic system. Buick (1992)

inferred from stromatolites in the Tumbiana formation of the late Archean located in Western Australia that oxygenic photosynthesis occurred about 2.7 billion years ago.

Stromatolites became common in the Proterozoic as the cyanobacteria achieved dominance as carbon-fixing and oxygen-evolving microorganisms. They formed as a result of the aggregation of some filamentous forms into mats that trapped siliceous and carbonaceous sediment, which in many instances contributed to their ultimate preservation by silicification. Environmental conditions at this time seemed to favor the mat-forming growth habit. Continental emergence, development of shallow seas, and climatic and atmospheric changes resulting from oxygenic photosynthesis probably exerted selective pressure favoring this growth habit of cyanobacteria (Knoll and Awramik, 1983). Most microfossil finds representing this period have been stromatolites, possibly because they are among the more easily recognized microfossils. Unicellular or inconspicuous multicellular microfossils would be much harder to find and identify, so one should not draw the conclusion that mat-forming cyanobacteria were necessarily the only common form of life at the time.

Initially the oxygen that was evolved in oxygenic photosynthesis probably reacted with oxidizable inorganic matter such as iron [Fe(II)], forming iron oxides such as magnetite (Fe_3O_4) and hematite (Fe_2O_3) (see Chap. 15). But eventually, about 2.3 billion years ago (Schopf, 1978) or a little earlier according to more recent estimates, free oxygen began to accumulate in the atmosphere, gradually changing it from a nonoxidizing atmosphere to a distinctly oxidizing one. As the atmospheric oxygen concentration increased, some organisms began to evolve biochemical catalysts and other molecules capable of electron transfer that included **cytochromes**, which are proteins containing iron porphyrins that could have arisen from the magnesium porphyrins of the chlorophylls of photosynthetic prokaryotes. The cytochromes were incorporated into electron transport systems in the plasma membrane, thereby enabling cells to dispose of excess reducing power (electrons) to molecular oxygen instead of partially reduced organic compounds (see Chap. 6). The cytochrome system made possible a reaction sequence of discrete steps (*respiratory chain*), in some of which metabolic energy could be conserved by trapping it in special chemical (anhydride) bonds for subsequent utilization in energy-requiring reactions (syntheses, polymerizations) (see Chap. 6). Because the biochemical oxygen reduction process leads to the formation of very toxic superoxide radicals (O_2^-) (Fridovich, 1977), the oxygen-utilizing organisms had to evolve a special protective system against them. Such a system included **superoxide dismutase** and **catalase**, metalloenzymes that together catalyze the reduction of superoxide to water and oxygen. Superoxide dismutase catalyzes the reaction

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \tag{3.6}$$

and catalase catalyzes the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2 \tag{3.7}$$

Peroxidase may replace catalase as the enzyme for disposing of the toxic hydrogen peroxide,

$$2RH + H_2O_2 \rightarrow 2H_2O + 2R \tag{3.8}$$

where RH represents an oxidizable organic molecule.

Schopf et al. (1983) suggested that the first oxygen-utilizing prokaryotes were *amphiaerobic*; i.e., they retained the ability to live anaerobically even though they had acquired the ability to metabolize aerobically. (Present-day amphiaerobes are called **facultative** organisms.) From them, more than 2 billion years ago, the obligate aerobes evolved, according to Schopf et al. (1983). Towe (1990) proposed that aerobes could have first appeared in the Early Archean. In any case, aerobes became well established only around 2 billion years ago. The evolutionary sequence leading to aerobes was probably complex, because present-day facultative prokaryotes include some that have a respiratory system that can use nitrate, ferric iron, manganese oxides, and/or some other oxidized inorganic species as terminal electron acceptors in place of oxygen. Such respiratory systems could not have existed before the atmosphere became oxidizing, because, except for ferric iron, most of these electron acceptors would not have occurred in sufficient quantities in a reducing atmosphere.

Amphiaerobes that evolved in the late Archean to Early Proterozoic are best defined as organisms that had the capacity to *respire* aerobically in the presence of oxygen or to *ferment* in its absence. The methanogens are an exception. They first appeared much earlier. Although strict anaerobes, they *respired* anaerobically using CO_2 as the terminal electron acceptor. The sulfate reducers are another exception. They are obligate anaerobes that respire on SO_4^{2-} (some sulfate reducers are now known that can reduce sulfate aerobically as well but cannot grow in this mode; see Chap. 18 for details).

Some strict anaerobes probably also evolved subsequent to the appearance of oxygen in the atmosphere. These were organisms with a capacity to respire anaerobically. The reason for this evolutionary development was that atmospheric oxygen must have led to extensive accumulation of oxidized species such as sulfate and others mentioned above, which could serve as terminal electron acceptors in a respiratory process. Sulfate reducers in the domain Bacteria have been viewed as having made their first appearance in the Late Archean (Ripley and Nicol, 1981). They have been important ever since their first appearance in the reductive segment of the sulfur cycle in the mesophilic temperature range (~15–40°C) (Schidlowski et al., 1983). At ambient temperature and pressure, bacterial sulfate reduction is the only process whereby sulfate can be reduced to hydrogen sulfide. A group of extremely thermophilic, archaeal sulfate reducers

were isolated more recently from marine hydrothermal systems in Italy (Stetter et al., 1987), which in the laboratory grew in a temperature range of 64–92°C with an optimum near 83°C. They reduce sulfate, thiosulfate, and sulfite with H_2, formate, formamide, lactate, and pyruvate. According to Stetter et al. (1987), these types of bacteria may have inhabited Early Archean hydrothermal systems containing significant amounts of sulfate of magmatic origin.

The accumulation of oxygen in the atmosphere led to the buildup of an ozone (O_3) shield that screened out UV components of sunlight. The ozone results from the effect of short-wavelength UV on O_2:

$$3O_2 \rightarrow 2O_3 \tag{3.9}$$

The ozone screen would have stopped any abiotic synthesis dependent on UV irradiation and at the same time would have allowed the emergence of life forms onto land surfaces of Earth, where they were directly exposed to sunlight. This emergence would have been impossible earlier because of the lethality of UV radiation (Schopf et al., 1983).

With the appearance of oxygen-producing cyanobacteria and aerobic heterotrophs, the stage was set for cellular compartmentalization of such vital processes as photosynthesis and respiration. New types of photosynthetic cells evolved in which photosynthesis was carried on in special organelles, the **chloroplasts**, and new types of respiring cells evolved in which respiration was carried on in other special organelles, the **mitochondria**. It is now generally believed that these organelles arose by endosymbiosis. This was a process in which primitive cells that were incapable of photosynthesis or respiration were invaded by cyanobacteria-like organisms (e.g., *Prochloron*, which contains both chlorophyll a and b like most chloroplasts, whereas other cyanobacteria contain only chlorophyll a) and/or by aerobically respiring bacteria. These invading organisms established a permanent symbiosis with their host (see discussion by Margulis, 1970; Dodson, 1979). The genetic apparatus in the host cells became enveloped in a double membrane by an as yet unknown mechanism. Eventually the relationship of some of the host cells with their endosymbionts became one of absolute interdependence, the endosymbionts having lost their capacity for an independent existence. The result was the first appearance of **eukaryotic cells**, possibly as late as 1 billion years ago (Schopf et al., 1983) but more likely as early as 2.1 billion years ago (Han and Runnegar, 1992). The highly compartmentalized organization of these cells contrasts with the much less compartmentalized organization of **prokaryotic** cells (Bacteria including cyanobacteria, and Archaea).

Supporting evidence for an endosymbiotic origin of chloroplasts and mitochondria in eukaryotic cells is the discovery in these organelles of genetic substance—deoxyribonucleic acid—and of cell particles called ribosomes, each of a type that is otherwise found only in prokaryotes. Furthermore, in the case of

chloroplasts, molecular biological comparisons of the highly conserved 16S ribonucleic acid fraction in some cyanobacterial and chloroplast ribosomes have revealed close relatedness to bacteria (Giovannoni et al., 1988). The emergence of eukaryotic cells was evidently needed for the evolution of more complex forms of life, such as the protozoa, fungi, plants, and animals.

Early Evolution According to the Surface Metabolist Scenario

If surface metabolism as described by Wächtershäuser (1988) preceded cellular metabolism, the description of the beginning of life and its early evolution requires an extensive revision of the organic soup theory scenario. Wächtershäuser's surface metabolist scenario is an autocatalytic process that does not involve enzymes or templates for their formation. Any dissolved organic molecules synthesized abiotically with heat, electric, or radiant energy did not play a role in surface metabolism as conceived by him. All organic molecules formed by metabolists were the result of interactions between inorganic species on positively charged mineral surfaces. Pyrite (FeS_2) surfaces were favored by Wächtershäuser. The metabolism was *autotrophic* in that the starting molecules (reactants) were CO_2, CO, NH_3 (or N_2 after emergence of nitrogen fixation), H_2S, H_2, and H_2O. Detachment of metabolists from the positively charged mineral surface took place after they became enveloped in a lipid membrane, also formed on the positively charged mineral surface. Each membrane enclosed a cytosol in which enzymes and templates for replicating them appeared gradually with the development of a genetic apparatus. But until the advent of enzymes and templates in these membrane vesicles, surface metabolism was still central to continued life. As in the precellular state, it took place on a mineral grain with a positively charged surface, but now one located in the cytosol. The grains were still very likely iron pyrite, formed in energy metabolism involving the interaction of ferrous iron with hydrogen sulfide [see Sec. 3.1, Eq. (3.1)]. After the appearance of enzymes in the cytosol, cells gradually dispensed with surface metabolism on the intracellular mineral particle, probably by shifting to energy-yielding reactions that did not form the particles (see below).

In Wächtershäuser's view (1988), the cytosol and the appearance of enzymes in it made possible for the first time the utilization of intracellularly accumulated, surface-detached reaction products (salvaging) that were not needed for biosynthesis. Because the first cells were autotrophs, this represents a form of *autotrophic* **catabolism**. The evolution of a membrane-bound respiratory system, which followed, was a consequence of the development of the cell membrane. According to Wächtershäuser (1988), the respiratory chain freed organisms that developed it from the need to use the reaction of ferrous iron with H_2S as a source of energy and reducing power. They were now able to use a reaction such as one

in which H_2 reduces elemental sulfur (S^0) or sulfate to hydrogen sulfide. Wächtershäuser also saw autotrophic catabolism as a point of departure for the evolution of substrate-level phosphorylation (see Chap. 6) as an alternative means of conserving energy. He believes that heterotrophy evolved from autotrophic catabolism in cells that now developed a transport mechanism for importing dissolved organic molecules from the surrounding environment. He believes that this occurred at a late stage in the evolution of early cells. The first prokaryotic anoxygenic photosynthesizers probably appeared some time before the first heterotrophs appeared. Thus, in the surface metabolism scenario, autotrophy preceded heterotrophy, and anaerobic chemosynthetic autotrophy preceded anoxygenic photosynthetic autotrophy.

3.3 THE EVIDENCE

The scenarios for the origin and early evolution of life outlined in the previous section are at best based on educated guesses. Scenarios for the evolution of cellular life have been constructed in part on the basis of observations in the geologic record and in part on the basis of comparisons between fossilized microorganisms and their present-day counterparts. The geologic record has contributed supporting evidence in the form of microfossils and geochemical data. Morphological similarities between microfossils and certain present-day organisms as well as some geochemical data relating to the site where the fossilized organisms were found have permitted inferences to be drawn concerning likely physiological and biochemical activities of the fossilized organisms when they were alive. Molecular biological analysis of present-day organisms has permitted the construction of evolutionary trees that reflect biochemical evolution and permit an estimate of the time of first appearance of the organisms, using as a basis the conservation of certain genetic information over geologic periods of time (Woese, 1987; Olsen et al., 1994).

The identification of Precambrian microfossils is difficult because structures that appear to be fossilized microorganisms may actually be modern contaminants or abiotically formed structures resembling microfossils in appearance. A true microfossil should meet the following criteria (Schopf and Walter, 1983):

1. The sedimentary rock in which the microfossil was found must be of a scientifically established age.
2. The purported fossil must be indigenous to the rock sample and not a modern contaminant.
3. The purported fossil must have been formed at the same time as the enclosing rock, i.e., **syngenetically**.
4. The purported fossil must have had a true biogenic origin.

On the basis of these criteria, some previously identified microfossils have had to be rejected as bona fide and reclassified as *dubiofossils* (uncertainty about biogenicity), *nonfossils* (may be present-day microbes that invaded a particular rock in situ), or **contaminants** introduced during sampling. Walter (1983) and Hofmann and Schopf (1983) reclassified Precambrian fossil finds before 1983 into the above categories based on the criteria of Schopf and Walter (1983).

Microfossils arose as a result of entrapment of microorganisms in siliceous sediment. This was followed by impregnation of cell structures like those of cyanobacteria with dissolved silica from mineral diagenesis. Subsequent dewatering under conditions of moderately elevated temperature and pressure resulted in precipitation of silica in the cells. This process has been reproduced in the laboratory on a time scale obviously compressed over many orders of magnitude (Oehler and Schopf, 1971). Fossilization of bacteria may also have involved the concentration of certain metallic ion species in their cell envelope and subsequent crystallization of a specific sulfide, phosphate, oxide, carbonate, silicate, or other mineral from the accumulated metal (Beveridge et al., 1983; Ferris et al., 1986). Observations by Ferris et al. (1986) suggest that microfossils formed as a result of mineral precipitation were probably best preserved if they had been previously embedded in a fibrous silica matrix (*premineralization*). Growth of crystals of metal-containing minerals would otherwise have caused rupture of the cells. Microfossils are visualized by thin-sectioning sedimentary rock containing them and examining such sections, after suitable treatment, by light and/or electron microscopy.

Bona fide Archean microfossils are represented by Warrawoona specimens (~3.5 billion years old) from the North Pole Dome region of the Pilbara Block in Western Australia (Fig. 3.1), in which have been found examples of "filamentous bacteria" (Fig. 3.2) (see Schopf and Walter, 1983) and microfossiliferous stromatolites (Walter, 1983). Unicell-like spheroids, which are currently classified as dubiofossils, have also been seen in these specimens (see Schopf and Walter, 1983). Spheroid structures of an age similar to the Warrawoona specimens, classified as dubiofossils, have been found in the Onverwacht Group of the Swaziland System, eastern Transvaal, South Africa. Filamentous fossil bacteria, ~2.7 billion years old, have been identified in specimens of the Fortescue Group, Tumbiana Formation in Western Australia (see Schopf and Walter, 1983).

Bona fide Proterozoic microfossils have been identified in greater numbers than Archean microfossils. They include filamentous organisms named *Gunflintia minuta* Barghoorn in Pokegama quartzite (1.8–2.1 billion years old), coccoid, septate filamentous, and tubular unbranched and budding bacteria-like microfossils in the Gunflint Formation of northwestern Ontario, Canada (1.8–2.1 billion years old); coccoid, septate filamentous, and tubular, unbranched microfossils in the Tyler Formation of Gogebic County, northern Michigan (1.6–2.5 billion years old); spheroidal, planktonic organic walled microfossils in the

Krivoirog Series of the Ukranian Shield; and a number of other examples from other parts of the world (Fig. 3.3). Many of these microfossils were associated with stromatolites, which abounded in the Proterozoic (1.7–0.57 billion years ago) (Krylov and Semikhatov, 1976). These stromatolites were formed chiefly by cyanobacteria. Their abundance in the Proterozoic is attributed to environmental conditions that favored formation of cyanobacterial mats and the absence of organisms capable of grazing on such mats (Knoll and Awramik, 1983). With the emergence of grazers in the Phanerozoic, stromatolites became rare and have

FIG. 3.3 *Gunflintia* (?) sp.: A septate filament with unusually elongate cells, preserved in dark brown organic matter from Duck Creek Dolomite, Mount Stuart area, Western Australia: ~2.02 billion years old. Scale mark on right is 10 μm. (Reproduced from photo 14-3 J in Schopf, 1983, with publisher's permission.)

remained so to the present day. Modern stromatolites are confined to very special locations.

The finding of microfossils in Precambrian sedimentary rock formations is evidence of the presence of life at this time. Because of the resemblance of some of these microfossils to present-day cyanobacteria, inferences can be drawn concerning the physiology and biochemistry of these microfossils. It can be inferred, for instance, that photosynthesis, possibly oxygenic photosynthesis, occurred at the time corresponding to the geologic age of these particular fossils. On the other hand, if independent geochemical data indicate that a reducing or at least oxygen-lacking atmosphere prevailed at the time, the fossils may represent anoxic precursors of oxygenic cyanobacteria, or they may represent the time of emergence of oxygenic cyanobacteria.

No microfossils of the very earliest life forms have been found, nor are they likely ever to be found on Earth because of the weathering or diagenetic processes to which sedimentary rock on our planet has been subjected from the start and to which it is continuing to be subjected. This weathering was originally a physicochemical process involving water and various reactive substances in the planet's atmosphere. With the emergence of prokaryotic life, microbes also became important agents of weathering. Their present-day weathering activities are discussed in Chapters 4 and 9.

Geochemical studies of Precambrian rocks can also tell us something about early life. For instance, measurements of stable isotope ratios of major elements important to life, namely C, H, N, and S (Schidlowski et al., 1983) and perhaps even Fe (Beard et al., 1999; but see also Mandernack et al., 1999 and Anbar et al., 2000), can give an indication of whether a biological agent was involved in their formation or transformation. Such interpretation rests on observations that some present-day microorganisms can discriminate among stable isotopes by metabolizing the lighter species faster than the heavier species. Thus under appropriate conditions, they attack ^{12}C more readily than ^{13}C, hydrogen more readily than deuterium, ^{14}N more readily than ^{15}N, ^{32}S more readily than ^{34}S, or ^{54}Fe more readily than ^{56}Fe. Abiotic reactions do not discriminate among stable isotopes to this extent. As a result, products of microbial fractionation reactions will show an enrichment with respect to the lighter isotope. Residual substrates will show an enrichment in the heavier isotope. This occurs in initial stages of a reaction in a closed system or in an open system with a low rate of substrate consumption (Chap. 6). Carbon isotope studies of many sediment samples of Archean age indicate that life played a dominant role in the carbon cycle as far back as 3.5 billion years ago (Schidlowski et al., 1983). Sulfur isotopic data for Early Archean sediments indicate the likely activity of photosynthetic bacteria [barites ($BaSO_4$) of this time were only slightly enriched in ^{34}S compared to sulfides from the same sequence (Schidlowski et al., 1983)]. They also suggest that sulfate respiration by prokaryotes may not have occurred to a significant extent before

2.7 billion years ago. Some more recent geochemical evidence indicates, however, that some sulfate reduction occurred as long ago as 3.4 billion years (Ohmoto et al., 1993).

Organic geochemistry provides another approach to seeking clues to early life on Earth. Organic matter trapped in sediment subjected to abiological transformation due to heat and pressure may be transformed into products that are stable in situ over geologic time. Organic matter that underwent this kind of transformation is likely to have been in a form that was not rapidly degraded by biological means as are carbohydrates, nucleic acids, and nucleotides and most proteins. Nevertheless, amino acids, fatty acids, porphyrins, *n*-alkanes, and isoprenoid hydrocarbons have been identified in sediments of Archean age (Kvenvolden, cited by Schopf, 1977; Hodgson and Whiteley, 1980). If the source compound of any stable organic product identified in an ancient sample of sedimentary rock is known, the latter can be used as an indicator or "biological marker" of the source compound. If the source compound such as porphyrin is a key compound in a particular physiological process, it indicates that a process like photosynthesis or respiration or both was occurring when the source material became trapped in sediment. *Kerogen* is an example of a stabilized substance formed from ancient organic matter. Its presence in an ancient sedimentary rock suggests the existence of life contemporaneous with the age of that rock.

Studies in molecular biology have revealed that the proportion and sequence of certain monomers in some bioheteropolymers such as ribosomal RNAs are highly conserved in various organisms, i.e., they have not become significantly modified over very long times due to extremely slow mutation rates. Such conserved sequences can be used to study the degree of relatedness among different groups of organisms (e.g., see Fox et al., 1980; Woese, 1987; Olsen et al., 1994) and can also be used to estimate the geologic time at which they first appeared. Such studies have led to the conclusion that Archaea (formerly called Archaebacteria and Archaeobacteria) and Bacteria (formerly called Eubacteria) diverged early in Archean times from a common prokaryotic ancestor and have evolved ever since along independent parallel lines. They also indicate that gram-positive bacteria most likely had a photosynthetic ancestry (Woese et al, 1985). The fact that certain physiological processes such as protein synthesis, energy conservation by chemiosmosis, and some biodegradative as well as biosynthetic pathways are held in common by the Bacterial and Archaean domains although differing in some details, suggests that these pathways may have existed in a common ancestor but became modified during divergent evolution. Convergent evolution cannot, however, be ruled out in all cases.

Combining several lines of paleontological evidence can lend strong support for a model of an ancient biological process and/or microbe responsible for it. Summons and Powell (1986) found in a certain Canadian petroleum deposit of Silurian age ($\sim 400 \times 10^6$ years ago) the presence of (1) characteristic biological markers indicating an ancient presence of aromatic carotenoids from

green sulfur bacteria (Chlorobiaceae) and (2) enrichment in ^{13}C in these markers relative to the saturated oils. Relating these findings to the paleoenvironmental setting of the oil deposit, the investigators deduced that microbial communities that included Chlorobiaceae must have existed in the ancient restricted sea in which the source material from which the oil derived was emplaced.

3.4 SUMMARY

The Earth is about 4.6 eons old. Primitive life probably arose de novo, first appearing 0.5–0.7 eon after formation of the planet. Initially, Earth was surrounded by an atmosphere that lacked oxygen and may have been reducing or nonreducing. Oxygen did not start to accumulate in the atmosphere until oxygen-generating, photosynthetic microorganisms, the cyanobacteria, had evolved and become established and oxygen-scavenging entities like ferrous iron had been depleted by reacting with the evolved oxygen. The time at which oxygen began to accumulate is currently controversial. A conservative estimate is 2.3 eons ago, but it may have been earlier.

The earliest forms of cellular life were anaerobic prokaryotes. Except for cyanobacteria, aerobic prokaryotes did not evolve until free oxygen began to accumulate in the atmosphere, and eukaryotic forms did not appear until the accumulated oxygen in the atmosphere attained significant levels, about 2.1 eons ago.

The evolutionary sequence of prokaryotes in terms of physiological types according to the organic soup scenario started with fermenting heterotrophs and progressed with the development of anaerobic photo- and chemoautotrophs, some anaerobic respirers, oxygenic photoautotrophs, aerobically respiring heterotrophs and autotrophs, and other anaerobic respirers. Eukaryotes evolved by endosymbiosis involving anaerobic heterotrophic prokaryotes as host cells and oxygenically photosynthetic and aerobically respiring prokaryotes as intracellular symbionts. According to the surface metabolist theory, the sequence of emergence of prokaryotic physiological types was autotrophic surface metabolists (precellular); semicellular surface metabolists; membrane-bound, detached, chemosynthetically autotrophic primitive cells; anoxygenic photosynthetic autotrophs; and aerobically respiring heterotrophs; followed by the emergence of eukaryotes. Major steps in Precambrian evolution according to the organic soup scenario and the surface metabolism scenario are summarized in Table 3.2.

Aspects of currently held views of how life evolved on Earth are supported by the microfossil record in geologically dated sedimentary rock, by inorganic and organic geochemical studies of Precambrian rocks, and by molecular biological analysis of highly conserved polymers such as some nucleic acids and proteins from living cells.

TABLE 3.2 Milestones in Precambrian Evolution of Life

Event	Years before present[a]
A. Organic soup scenario	
Origin of the Earth	4.6×10^9
First self-reproducing molecules	$4.3–4.0 \times 10^9$ (?)
First primitive heterotrophic cells	$4.0–3.8 \times 10^9$ (?)
First autotrophs (methanogens and/or anoxygenic photosynthesizers)	$\sim 3.8 \times 10^9$ (?)
Warrawoona stromatolite	$\sim 3.5 \times 10^9$
First oxygenic photosynthesizers	$3.5–3.0 \times 10^9$
First anaerobic respirers (sulfate reducers)[b]	$\sim 2.7 \times 10^9$
Fully oxidizing atmosphere	$\sim 2.1 \times 10^9$
First aerobic respirers[c]	$\sim 2.0 \times 10^9$
First eukaryotic cells	$\sim 1.4 \times 10^9$
B. Surface metabolism scenario[d]	
Origin of the Earth	4.6×10^9
First surface metabolists (chemoautotrophic)	$\sim 4.3 \times 10^9$ (?)
First primitive cells (chemoautotrophic)	$\sim 4.1 \times 10^9$ (?)
First anaerobic S and SO_4 respirers (autotrophic)	$\sim 4.0 \times 10^9$ (?)
First autotrophs	$\sim 3.8 \times 10^9$ (?)
First heterotrophs	$\sim 3.5 \times 10^9$ (?)
Warrawoona stromatolite	3.5×10^9
First oxygenic photosynthesizers	$\sim 3.5–3.0 \times 10^9$ (?)
Fully oxidizing atmosphere	$\sim 2.1 \times 10^9$
First aerobic respirers	$\sim 2.0 \times 10^9$
First eukaryotic cells	$\sim 1.4 \times 10^9$

[a] Dates followed by (?) represent guesses without any paleontological or molecular evolutionary backup.
[b] Most known sulfate reducers belong to the domain Bacteria. The discovery of sulfate-reducing Archaea (e.g., *Archeoglobus fulgidus*) suggests that some bacterial sulfate reduction may have occurred earlier.
[c] According to some students of early evolution, aerobic respirers could have evolved earlier than this.
[d] Sequence in scenario B is based on evolutionary descriptions of Wächtershäuser (1988).

REFERENCES

Anbar AD, Roe JE, Barling J, Nealson KH. 2000. Nonbiological fractionation of iron isotopes. Science 288:126–128.

Bada JL, Bingham C, Miller SL. 1994. Impact melting of frozen oceans on the early Earth: Implications for the origin of life. Proc Natl Acad Sci USA 91:1248–1250.

Beard BL, Johnson CM, Cox L, Sun H, Nealson KH, Aguillar C. 1999. Iron isotope biosignatures. Science 285:1889–1892.

Beveridge TJ, Meloche JD, Fyfe WS, Murray RGE. 1983. Diagenesis of metals chemically complexed to bacteria: Laboratory formulation of metal phosphates, sulfides, and organic condensates in artificial sediments. Appl Environ Microbiol 45:1094–1108.

Broad WJ. 1997. Spotlight on comets in shaping of Earth, Science Section, NY Times, June 3, 1997.

Buick R. 1992. The antiquity of oxygenic photosynthesis: Evidence from stromatolites in sulfate-deficient Archean lakes. Science 255:74–77.

Cairns-Smith AG, Hartman H, eds. 1986. Clay Minerals and the Origin of Life. Cambridge, UK: Cambridge Univ Press.

Cech TR. 1986. A model for the RNA-catalyzed replication of RNA. Proc Natl Acad Sci USA 83:4360–4363.

Chakrabarti AC, Breaker RR, Joyce GF, Deamer DW. 1994. Production of RNA by a polymerase protein encapsulated within phospholipid vesicles. J Molec Evol 39:555–559.

Chang S, DesMarais D, Mack R, Miller SL, Strathearn GE. 1983. Prebiotic organic syntheses and the origin of life. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 53–92.

Cohen J. 1995. Getting all turned around over the origins of life on Earth. Science 267:1265–1266.

Cohen Y, Padan E, Shilo M. 1975. Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. J Bacteriol 123:855–861.

Delsemme AH. 2001. An argument for the cometary origin of the biosphere. Am Sci 89:432–442.

Des Marais DJ. 2000. When did photosynthesis emerge on Earth? Science 289:1703–1705.

Dodson EO. 1979. Crossing the prokaryote–eukaryote border: Endosymbiosis or continuous development. Can J Microbiol 25:651–674.

Doebler SA. 2000. The dawn of the protein era. BioScience 50:15–20.

Doudna JA, Szostak JW. 1989. RNA-catalyzed synthesis of complementary-strand RNA. Nature (Lond) 339:519–522.

Drobner E, Huber H, Wächtershäuser G, Rose D, Stetter KO. 1990. Pyrite formation linked with hydrogen evolution under anaerobic conditions. Nature (Lond) 346:742–744.

Ernst WG. 1983. The early Earth and the Archean rock record. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 41–52.

Ferris FG, Beveridge TJ, Fyfe WS. 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. Nature (Lond) 320:609–611.

Fox GE, Magrum LJ, Balch WE, Wolfe RS, Woese CR. 1977. Classification of methanogenic bacteria by 16S ribosomal RNA characterization. Proc Natl Acad Sci USA 74:4537–4541.

Fox GE, Stackebrandt E, Hespell RB, Gibson J, Maniloff T, Dyer A, Wolfe RS, Balch WE, Tanner RS, Magrum LJ, Zablen LB, Blakemore R, Gupta R, Bonen L, Lewis BJ, Stahl

DFA, Luehrsen KR, Chen KN, Woese CR. 1980. The phylogeny of prokaryotes. Science 209:457–463.
Fridovich I. 1977. Oxygen is toxic! BioScience 27:462–466.
Gilbert W. 1986. The RNA world. Nature (Lond) 319:618.
Giovannoni SJ, Turner S, Olsen GJ, Barns S, Land DJ, Pace NR. 1988. Evolutionary relationships among cyanobacteria and green chloroplasts. J Bacteriol 170:3584–3592.
Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S. 1983. The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell 35:849–857.
Haldane JBS. 1929. The origin of life. Rationalist Annu 1929:148–169. (Reprinted in Smith JM, ed. 1985. On Being the Right Size and Other Essays. Oxford, UK: Oxford Univ Press, pp 101–112.)
Han T-M, Runnegar B. 1992. Megascopic eukaryotic algae from the 2.1-billion-year-old Negaunee iron-formation, Michigan. Science 257:232–235.
Hodgson GW, Whiteley CG. 1980. The universe of porphyrins. In: Trudinger PA, Walter MR, Ralph BJ, eds. Biogeochemistry of Ancient and Modern Environments. Canberra, Australia: Australian Acad Sci, pp 35–44.
Hofman HJ, Schopf JW. 1983. Early Proterozoic microfossils. In: Schopf WJ, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 321–360.
Holland HD. 1997. Evidence for life on Earth more than 3850 million years ago. Science 275:38–39.
Joyce GF. 1991. The rise and fall of the RNA world. New Biol 3:399–407.
Knoll AH, Awramik SM. 1983. Ancient microbial ecosystems. In: Krumbein WE, ed. Microbial Geochemistry. Oxford, UK: Blackwell Scientific, pp 287–315.
Kruger K, Grabowski PJ, Zaug AJ, Sands J, Gottschling DE, Cech TR. 1982. Self-splicing RNA: Autoexcision and autocatalyzation of the ribosomal RNA intervening sequence of *Tetrahymena*. Cell 31:147–157.
Krylov IN, Semikhatov MA. 1976. Appendix II. Table of time ranges of the principal groups of Precambrian stromatolites. In: Walter MR, ed. Stromatolites. Amsterdam: Elsevier, pp 693–694.
Lowe DR. 1980. Stromatolites 3,400 Myr old from the Archean of Western Australia. Nature (Lond) 284:441–443.
Mandernack KW, Bazylinski DA, Shanks WC III, Bullen TD. 1999. Oxygen and iron isotope studies of magnetite produced by magnetotactic bacteria. Science 285:1892–1896.
Margulis L. 1970. Origin of Eukaryotic Cells. New Haven, CT: Yale Univ Press.
Miller SL, Orgel LE. 1974. The Origins of Life on Earth. Englewood Cliffs, NJ: Prentice-Hall.
Mojzsis SJ, Arrhenius G, McKeegan KD, Harrison TM, Nutman AP, Friend CRL. 1996. Evidence for life on Earth before 3800 million years ago. Nature (Lond) 384:55–59.
Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P. 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 64:548–572.

Oehler JH, Schopf JW. 1971. Artificial microfossils: Experimental studies of permineralization of blue-green algae in silica. Science 174:1229–1231.
Ohmoto H, Kakegawa T, Lowe DR. 1993. 3.4 Billion-year-old biogenic pyrites from Barberton, South Africa: Sulfur isotope evidence. Science 262:555–557.
Olsen GJ, Woese CR, Overbeek R. 1994. The winds of evolutionary change: Breathing new life into microbiology. J Bacteriol 176:1–6.
Oparin AI. 1938. The Origin of Life. New York: Macmillan. (Reprinted by Dover Publications, Inc., New York, 1953.)
Orpen JL, Wilson JF. 1981. Stromatolites at ~3500 Myr and a greenstone-granite unconformity in the Zimbabwean Archean. Nature (Lond) 291:218–220.
Ripley EM, Nicol DL. 1981. Sulfur isotopic studies of Archean slate and graywacke from northern Minnesota: Evidence for the existence of sulfate reducing bacteria. Geochim Cosmochim Acta 45:839–846.
Russell MJ, Hall AJ. 1997. The emergence of life from iron monosulfide bubbles at a submarine hydrothermal redox and pH front. J Geol Soc Lond 154:377–402.
Schidlowski M, Hayes JM, Kaplan IR. 1983. Isotopic inferences of ancient biochemistries: Carbon, sulfur, hydrogen, and nitrogen. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 149–186.
Schopf JW. 1977. Evidences of Archean life. In: Ponnamperuma C, ed. Chemical Evolution of the Early Precambrian. New York: Academic Press, pp 101–105.
Schopf JW. 1978. The evolution of the earliest cells. Am Sci 238:110–139.
Schopf JW, ed. 1983. Earth's Earliest Biosphere: Its Origin and Evolution. Princeton, NJ: Princeton Univ Press.
Schopf JW, Packer BM. 1987. Early Archean (3.3-billion-year-old) microfossils from Warrawoona Group, Australia. Science 237:70–73.
Schopf JW, Walter MR. 1983. Archean microfossils: New evidence of ancient microbes. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 214–239.
Schopf JW, Hayes JM, Walter MR. 1983. Evolution of Earth's earliest ecosystems: Recent progress and unsolved problems. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 360–384.
Stetter KO, Lauerer G, Thomm M, Neuner A. 1987. Isolation of extremely thermophilic sulfate reducers: Evidence for a novel branch of Archaebacteria. Science 236:822–824.
Stevenson DJ. 1983. The nature of the Earth prior to the oldest known rock record: The Hadean Earth. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 32–40.
Summons RE, Powell TG. 1986. Chlorobiaceae in Paleozoic seas revealed by biological markers, isotopes, and geology. Nature (Lond) 319:763–765.
Taylor SR. 1992. The origin of the Earth. In: Brown G, Hawkesworth C, Wilson C, eds. Understanding the Earth. Cambridge, UK: Cambridge Univ Press, pp 25–43.
Towe KM. 1990. Aerobic respiration in the Archean. Nature (Lond) 348:54–56.
Wächtershäuser G. 1988. Before enzymes and templates: Theory of surface metabolism. Microbiol Rev 52:452–484.

Walter MR. 1983. Archean stromatolites: Evidence of the Earth's earliest benthos. In: Schopf JW, ed. Earth's Earliest Biosphere. Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 187–213.
Walter MR, Buick R, Dunlop JSR. 1980. Stromatolites 3,400–3,500 Myr old from the North Pole area, Western Australia. Nature 284:443–445.
Weber P, Greenberg JM. 1985. Can spores survive in interstellar space? Nature (Lond) 316:403–407.
Woese CR. 1987. Bacterial evolution. Microbiol Rev 51:221–271.
Woese CR, Fox GE. 1977. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. Proc Natl Acad Sci USA 74:5088–5090.
Woese CR, Debrukner-Vossbrinck BA, Oyaizu H, Stackebrandt E, Ludwig W. 1985. Gram-positive bacteria: Possible photosynthetic ancestry. Science 229:762–765.
Xiong J, Fischer WM, Inoue K, Nakahara M, Bauer CE. 2000. Molecular evidence for the early evolution of photosynthesis. Science 289:1724–1730.

4

The Lithosphere as a Microbial Habitat

4.1 ROCK AND MINERALS

To understand how the lithosphere supports the existence of microbes on and in it and how microbes influence the formation and transformation of its rocks and minerals, we must familiarize ourselves with some general chemical and physical features of rocks and minerals.

Geologically, the term **rock** refers to massive, solid, inorganic matter consisting usually of two or more intergrown minerals. Rock may be **igneous** in origin; that is, it may arise by cooling of **magma** (molten rock material) from the interior of the Earth (crust and/or asthenosphere). The cooling may be a slow process or a fast process. In slow cooling, different minerals begin to crystallize at different times, owing to their different melting points. In the process of crystallization, they intergrow and thereby evolve into rock with visually distinguishable crystals, as seen in granite, for example (Fig. 4.1). In fast cooling, rapid crystallization occurs, and the rock that forms contains only tiny crystals, not visible to the naked eye. Basalt is an example of rock formed in this way.

Rock may also be **sedimentary** in origin; that is, it may arise through the accumulation and compaction of sediment that consists mainly of mineral matter derived from other rock. In other instances, it may arise as a result of cementation of accumulated inorganic sediment by carbonate, silicate, aluminum oxide, ferric oxide, or a combination thereof. The cementing substance may result from

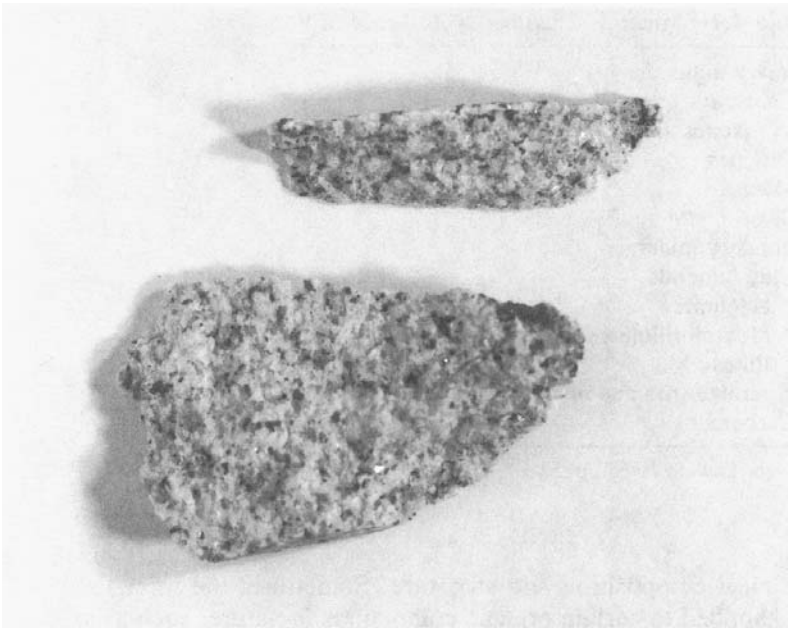


FIG. 4.1 Pieces of granite showing phenocrysts, i.e., visible crystals of mineral in a fine crystalline ground mass. An igneous rock. Fragment at top is 5 cm long.

microbial activity. These transformations of loose sediment into sedimentary rock are termed **lithification**. Examples of sedimentary rock are limestone, sandstone, and shale. Sedimentary rock facies often exhibit a layered structure in vertical section, reflecting changes in composition as the sediment is accumulated. Analysis of the different layers may tell something of the environmental conditions during which they accumulated.

Finally, rock may be of **metamorphic** origin; that is, it may be produced through alteration of igneous or sedimentary rock by the action of heat and pressure. Examples of metamorphic rock are marble, derived from limestone; slate, derived from shale; quartzite, derived from sandstone; and gneiss, derived from granitic rock.

Geochemically, *minerals* are usually defined as inorganic compounds, usually crystalline but occasionally amorphous, of specific chemical composition and structure. Sometimes the term "mineral" is also applied to certain organic compounds in nature, such as asphalt and coal. Inorganic minerals may be very simple in composition like sulfur (S^0) or quartz (SiO_2), or very complex, as in the case of the igneous mineral biotite [$K(Mg, Fe, Mn)_3AlSi_3O_{10}(OH)_2$]. Minerals

TABLE 4.1 Minerals Classified as to Mode of Formation

Primary minerals
Feldspars
Pyroxenes and amphiboles
Olivines
Micas
Silica
Secondary minerals
Clay minerals
Kaolinites
Montmorillonites
Illites
Hydrated iron and aluminum oxides
Carbonates

Source: Lawton (1955), p 54ff.

that result from crystallization during the cooling of magma are primary or *igneous* minerals. Minerals that result from chemical alteration (weathering or diagenesis) of primary minerals are known as *secondary* minerals. Microbes play a role in this transformation of primary to secondary minerals (Chap. 9). Examples of primary and secondary mineral groups are listed in Table 4.1. Minerals can also result from precipitation from solution, in which case they are called *authigenic* minerals. Microbes may also play a role in their formation (e.g., ferromanganese concretions; see Chap. 16).

4.2 MINERAL SOIL

Origin of Mineral Soil

The mineral constituents of mineral soil ultimately derived from rock that underwent weathering. Weathering that leads to soil formation is a process whereby rock is eroded and/or broken down into ever smaller particles and finally into constituent minerals. Some or all of these minerals may become chemically altered. Some forms of weathering involve physical processes. For example, freezing and thawing of water in cracks and crevices of rock may cause expansion of the rock from the pressure exerted by the ice because ice occupies a larger volume than an equivalent amount of water. Sand carried by wind may cause sandblasting of rock. Alternate heating by the sun's rays and cooling at night may also cause expansion and contraction of rock, leading to widening of

cracks and crevices. Waterborne abrasives or rock collisions may cause rock to break. Seismic activity may cause rock to crumble. Evaporation of hard water in cracks and fissures of rock and the resultant formation of crystals from the solutes may cause rock to break because the crystals occupy a larger volume than the original water solution from which they formed, thereby widening the cracks and fissures through the pressure they exert. Mere alternate wetting and drying may also cause rock breakup.

Rock weathering processes may also be chemical when the reagents are of nonbiological origin. Examples are the solvent action of water, CO_2 of volcanic origin, mineral acids such as H_2SO_3, HNO_2, and HNO_3 formed from gases of nonbiological origin such as SO_2, NO, and NO_2, respectively. Chemical weathering may also be caused by redox reagents of nonbiological origin, such as H_2S of volcanic origin or nitrate of atmospheric origin.

Finally, rock weathering may be the result of biological activity. Roots of plants penetrating cracks and fissures in rock may force it apart. Rock surfaces and the interior of porous rock are frequently inhabited by a flora of algae, fungi, lichens, and bacteria. The microorganisms on the surface of rocks may exist in biofilms, especially in a moist or wet environment. In biofilms containing a mixed microbial population, the different organisms may arrange themselves in distinct zones where conditions are most favorable for their existence (Costerton et al., 1994). Some microorganisms, so-called boring organisms, may form cavities in limestone rock that they occupy by causing dissolution of $CaCO_3$ (Golubic et al., 1975). In other cases, opportunistic microorganisms invade preformed cavities in rock (chasmolithic organisms) (Friedman, 1982). Invertebrates, snails in particular, may feed on boring organisms (Golubic and Schneider, 1979; Shachak et al., 1987) or chasmoliths by grinding away the superficial rock to expose them and then feeding on them. The rock debris that the snails generate then becomes part of a soil (Shachak et al., 1987; Jones and Shachak, 1990).

Microbes dissolve rock minerals through the production of reactive metabolic products such as NH_3, HNO_3, H_2SO_4 and CO_2 (forming H_2CO_3 in water) and oxalic, citric, and gluconic acids. Organic compounds formed by microorganisms such as lichens have been shown in studies using scanning electron microscopy to cause distinct weathering (Jones et al., 1981). Waksman and Starkey as long ago as 1931 cited the following reactions as examples of how microbes can affect weathering of minerals:

$$\underset{\text{orthoclase}}{2KAlSi_3O_8} + 2H_2O + CO_2 \rightarrow \underset{\text{kaolinite}}{H_4Al_2Si_2O_9} + K_2CO_3 + 4SiO_2 \tag{4.1}$$

$$\underset{\text{olivine}}{12MgFeSiO_4} + 26H_2O + 3O_2 \rightarrow \underset{\text{serpentine}}{4H_4Mg_3Si_2O_9} + 4SiO_2 + 6Fe_2O_3 \cdot 3H_2O \tag{4.2}$$

Reaction (4.1) is promoted by CO_2 production in the metabolism of heterotrophic microorganisms, and reaction (4.2) is promoted by O_2 production in photosynthesis, as by cyanobacteria, algae, and lichens inhabiting the surface of rocks. Recent investigations have extended these observations. Reactions were examined in which organic acids that are excreted by microbes promote weathering of primary minerals such as feldspars and secondary minerals such as clays (Browne and Driscoll, 1992; Lucas et al., 1993; Hiebert and Bennett, 1992; Welch and Ullman, 1993; Brady and Carroll, 1994; Oelkers et al., 1994; Ullman et al., 1996; Bennett et al., 1996; Barker and Banfield, 1996, 1998). Some current weathering models favor protonation as a means of displacing cationic components from the crystal lattice followed by cleaving of Si−O and Al−O bonds (e.g., Berner and Holdren, 1977; Chou and Wollast, 1984). Others favor complexation, for instance of Al and/or Si in aluminosilicates, as a primary mechanism of dissolution (Wieland and Stumm, 1992; Welch and Vandevivere, 1995).

Mineral soil may derive from aquatic sediment or *alluvium* left behind after the water that carried it from its place of origin to its final site of deposition has receded. Mineral soil can also form in place as a result of progressive weathering of parent rock and subsequent differentiation of weathering products. Soils originating by either mechanism undergo *eluviation* (removal of some products by washing out) and/or *alluviation* (addition of some products by water transport). Any soil, once formed, undergoes further gradual transformation due to the biological activity that it supports (Buol et al., 1980).

Some Structural Features of Mineral Soil

Mineral soil will vary in composition, depending on the source of the parent material, the extent of weathering, the amount of organic matter introduced into or generated in the soil, and the amount of moisture it holds. Its texture is affected by the particle sizes of its inorganic constituents (stones, >2 mm; sand grains, 0.05–2 mm; silt, 0.002–0.05 mm; clay particles, < 0.002 mm), which determine its porosity and this its permeability to water and gases.

Many, but not all, soils tend to be more or less obviously stratified. As many as three or four major strata or *horizons* may be recognizable in agricultural and forest soil *profiles*. A soil profile is a vertical section through soil (Fig. 4.2). The strata are called O, A, B, and C horizons. The O horizon represents the litter zone, consisting of much undecomposed and partially decomposed organic matter. It may be absent from a soil profile. The A and B horizons represent the true soil, and the C horizon represents the parent material from which the soil was formed. It may be bed rock or an earlier soil. The A and B horizons are often further subdivided, although these divisions are somewhat arbitrary. The A horizon is the biologically most active zone, containing most of the root systems of plants growing on it and the microbes and other life forms that inhabit soil. As is to be

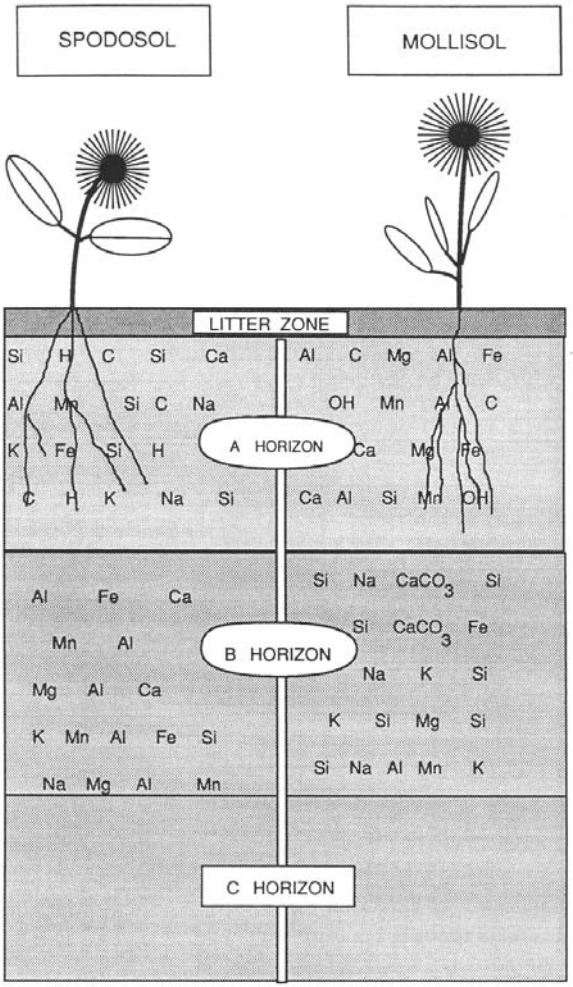


FIG. 4.2 Schematic representation of the major soil horizons of spodosol and mollisol. The litter zone is also called the O horizon. The A and B horizons may be further subdivided on the basis of soil chemistry.

expected, the carbon content is also highest in this horizon. The biological activity in the A horizon may cause solubilization of organic and inorganic matter, some or all of which, especially the inorganic matter is carried by soil water into the B horizon. The A horizon is therefore known at times as the *leached layer*, and the B horizon is at times known as the *enriched layer*. Both biological and abiological factors play a role in soil profile formation.

Effects of Plants and Animals on Soil Evolution

Biological agents such as plants assist soil evolution by contributing organic matter through excretions from their root systems and as dead organic matter. The plant excretions may react directly with some soil mineral constituents, or they may be modified together with the dead plant matter by microbial activity resulting in products that react with soil mineral constituents. During their lifetime, plants remove some minerals from soil and contribute to water movement through the soil by water absorption via their roots and transpiration from their leaves. Their root system may also help prevent destruction of the soil through wind and water erosion by anchoring it.

Burrowing invertebrates, from small mites to large earthworms, help to break up soil, keep it porous, and redistribute organic matter. The habitat of some of these invertebrates is restricted to specific regions of a soil profile.

Effects of Microbes on Soil Evolution

Microbes contribute to soil evolution by mineralizing some or all of any added organic matter during the decay process. Some of the metabolic products from this decay, such as organic and inorganic acids, CO_2 and NH_3, interact slowly with soil minerals and cause their alteration or solution, an important step in soil profile formation (Berthelin, 1977; Welch and Ullman, 1993; Ullman et al., 1996; Barker and Banfield, 1996, 1998). For instance, the mineral chlorite has been reported to be bacterially altered in this manner through loss or Fe and Mg and an increase in Si. The mineral vermiculite has been reported to be bacterially altered through solubilization of Si, Al, Fe, and Mg, thereby forming montmorillonite (Berthelin and Boymond, 1978). Certain microbes may interact directly (i.e., enzymatically) with certain inorganic soil constituents by oxidizing or reducing them (see Chaps. 12, 13, and 15–18) (Ehrlich, 2001), resulting in their solution or precipitation (Berthelin, 1977). Microbes may also play an important role in **humus** formation.

Humus is an important constituent of soil, consisting of humic and fulvic acids, humin and amino acids, lignin, amino sugars, and other compounds of biological origin (Paul and Clark, 1996, pp 148–152; Stevenson, 1994). Humic and fulvic acids are dispersible in solutions of NaOH or sodium pyrophosphate; humin is not. Humic acids are precipitated at acid pH whereas fulvic acids are not. These three constituents of humus represent components of soil organic matter that are only slowly decomposed. They are mostly formed by microbial attack of plant organic matter introduced into the litter zone (O horizon) and in the A horizon. Humus gives proper texture to soil and plays a significant role in regulating the availability of those mineral elements that are important in plant nutrition and in detoxifying those that are harmful to plants by complexing them. Humus also contributes to the water-holding capacity of soil. Some micro-

organisms in soil can use humic substances as terminal electron acceptors in anaerobic oxidation of organic compounds and H_2 and as electron shuttles in the anaerobic reduction of Fe(III) oxides (Lovley et al., 1996).

Effects of Water in Soil Evolution

Water from rain or melting snow may mobilize and transport some soluble soil components and cause precipitation of others. This can contribute to horizon development as the water permeates the soil. Precipitates, especially inorganic ones, may promote soil clumping. In addition, water may affect the distribution of soil gases by displacing the rather insoluble ones, such as nitrogen and oxygen, and by absorbing the more soluble ones, such as CO_2, NH_3, and H_2S.

Water Distribution in Mineral Soil

Only about 50% of the volume of mineral soil is solid matter. The other 50% is pore space occupied by water and by gases such as CO_2, N_2, and O_2. As might be expected, owing to the biological activity in soil and slow gas exchange with the external atmosphere, the CO_2 concentration in the gas space in soil usually exceeds that in air, whereas the O_2 concentration is less than that in air. According to Lebedev (see Kuznetsov et al., 1963, pp. 33–41), soil water may be distributed in distinct zones among soil particles (Fig. 4.3). Surrounding a soil particle is **hygroscopic water**, a thin film 3×10^{-2} μm in thickness when surrounding a 25 mm diameter particle. This water never freezes and never moves as a liquid. It is adsorbed by soil particles from water vapor in the atmosphere. In a water saturated atmosphere, **pellicular water** surrounds the hygroscopic water. Pellicular water may move from soil particle to soil particle by intermolecular attraction but not by gravity (Fig. 4.3B). It may contain dissolved salts, which may depress its freezing point to $-1.5°C$. **Gravitational water** surrounds pellicular water in Lebedev's model when moisture in excess of what the soil atmosphere can hold is present. It moves by gravity and responds to hydrostatic pressure, unlike hygroscopic water and pellicular water. So far, it is unclear which of these forms of water is available to microorganisms. A reasonable guess is that gravitational water and probably pellicular water can be used by them, but not hygroscopic water. The water need of microorganisms is usually studied in terms of moisture content, water activity, or water potential without regard to the form of soil water (Dommergues and Mangenot, 1970).

Water activity of a soil sample is a measure of the degree of water saturation of the vapor phase in the soil and is expressed in terms of relative humidity, but as a fractional number instead of percent. Pure water has a water activity of 1.0. Except for extreme halophiles, bacteria have a higher minimum water activity requirement (above 0.85) than many fungi (above 0.60) (Brock et al., 1984).

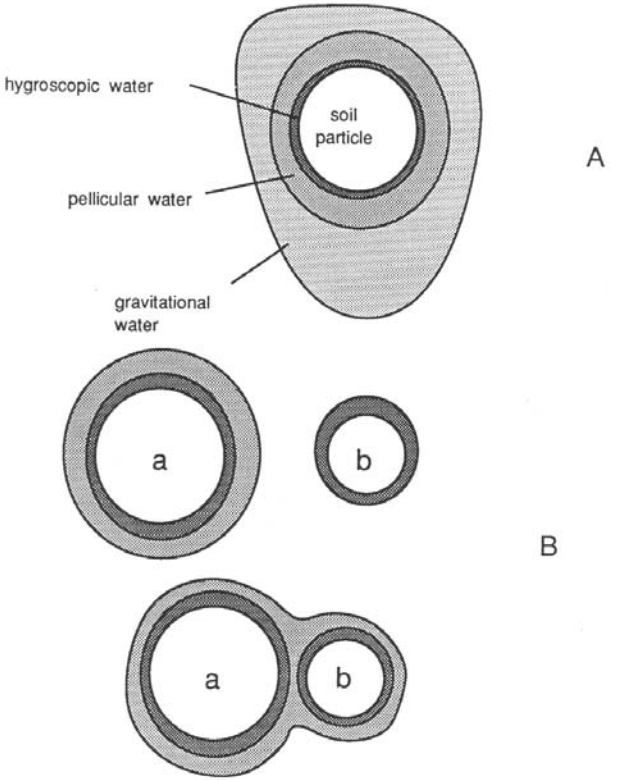


FIG. 4.3 Diagrammatic representation of soil water distribution according to Lebedev. For explanation of (A) and (B) see text. (Adapted from Kuznetsov et al., 1963.)

Water potential of a soil is a measure of water availability in terms of the difference between the free energy of the combined matrix and osmotic potentials of soil water and pure water at the same temperature. Matric effects on water availability have to do with the effect of water adsorption to solid surfaces such as soil particles, which lowers water availability. Osmotic effects have to do with the effect of dissolved solutes on water availability; their presence lowers it. Since matric and osmotic effects lower the free energy of water, water potential values are negative. The more negative the water potential value, the lower the water availability. A zero potential is equivalent to pure water. Osmotic water potential can be calculated from the effect of solute on the freezing point of water by using the formula of Lang (1967):

$$\text{Water potential (J kg}^{-1}) = 1.322 \times \text{freezing point depression} \qquad (4.3)$$

TABLE 4.2 Effect of Osmotic Water Potential (Glycerol) on Growth of *T. ferrooxidans*[a]

Glycerol	Total water potential (bars)	Strain 57-5[b]	Strain 59-1[b]
184	−61	−	−
147	−49	−	−
92	−32	−	−
74	−26	−	−
55	−20	−	−
37	−15	−	−
18.4	−8.8	+	−
9.2	−6	+	+
3.7	−4.2	+	+
0	−3	+	+

[a] All experiments were done in replicate tubes that showed the same results. The incubation period was 2 weeks. Iron concentration in the medium, 10 g of $FeSO_4 \cdot 7H_2O$ per liter.

[b] +, Visible iron oxidation and microscopically visible growth; −, no iron oxidation or microscopically visible growth.

Source: Brock (1975); reproduced with permission.

where 100 J kg^{-1} is equal to 1 bar. Matric water potential requirements can be determined by the method of Harris et al. (1970). In this method, NaCl or glycerol solutions of desired water potentials, solidified with agar, are used to equilibrate matrix material on which microbial growth is to occur. (For further discussion of water potential see Brock et al., 1984; Brown, 1976.)

The water potential requirement for two strains of the acidophilic iron oxidizer *Thiobacilllus ferrooxidans* has been determined by Brock (1975). Using NaCl as osmotic agent, strain 57-5 exhibited a minimum water potential requirement at −18 to −32 bar, whereas strain 59-1 exhibited it at −18 to −20 bar. Using glycerol as osmotic agent, strain 57-5 exhibited a minimum water potential at −8.8 bar, whereas strain 59-1 exhibited it at −6 bar (Table 4.2). In the same study, it was shown that significant amounts of CO_2 were assimilated by *T. ferrooxidans* on coal refuse material with water potentials between −8 and −29 bar, whereas none was assimilated when the water potential of the refuse was > -90 bar.

Nutrient Avaliability in Mineral Soil

Organic or inorganic nutrients required by soil microbes are distributed between the soil solution and the surface of mineral particles. Partitioning effects will determine their relative concentrations in the two phases. Their presence on the

surface of soil particles may be the result of adsorption or ion exchange. Nonionizable molecules will tend to be adsorbed, whereas ionizable ones will bind as a result of surface charges of opposite sign and may involve ion exchange. Microbial utilization of surface-bound molecules that are metabolized intracellularly may require either their displacement in order to be taken into the cells or, in the case of some polymeric organic molecules, direct attack, e.g., by hydrolysis, of the portion of the molecule that is not bound to the surface. If displacement or direct attack at the surface cannot be effected, such nutrients will be unavailable.

Clay particles are especially important in ionic binding of organic or inorganic cationic solutes (those having a positive charge). Such particles exhibit mostly negative charges except at their edges, where positive charges may appear. Their capacity for ion exchange depends on their crystal structure. The partitioning of solutes between soil solution and mineral surfaces often results in the greater concentration of solutes on mineral surfaces than in the soil solution, and as a result the mineral surfaces may be the preferred habitat of soil microbes that require these solutes in more concentrated form. On the other hand, ionically bound solutes on clay or other soil particles may be less available to soil microbes because the microbes may not be able to dislodge them from the particle surface. In that instance, soil solution may be the preferred habitat for microbes that have a requirement for such solutes. Ionic binding to soil particles may be beneficial if a solute subject to such binding is toxic and not readily dislodged (see Chap. 9).

Some Major Soil Types

Distinctive soil types may be identified by and correlated with climatic conditions and with the vegetation they support (Bunting, 1967; Buol et al., 1980). Climatic conditions determine the kind of vegetation that may develop. Thus, in the high northern latitudes, **tundra soil**, a type of *inceptisol*, prevails, which in that cold climate is often frozen and therefore supports only limited plant and microbial development. It has a poorly developed profile. It may be slightly acid to slightly alkaline. Examples of tundra soil are Arctic Brown Soil and Bog Soil. In the cool (i.e., temperate), humid zones at midlatitudes, **spodosols** (Figs. 4.2, 4.4) prevail, which support extensive forests, particularly of the coniferous type. Spodosols tend to be acidic, having a strongly leached, grayish A horizon depleted in colloids, iron, and aluminum and a brown B horizon enriched in iron, aluminum, and colloids leached from the A horizon. In regions of moderate rainfall in temperate climates at midlatitudes, mollisols (Figs. 4.2, 4.5) prevail. These are soils that support grasslands (i.e., they are prairie soils). They exhibit rich black topsoil and show lime accumulation in the B horizon because they have neutral to alkaline pH. **Oxisols** are found at low latitudes in tropical, humid climates. They are poorly zonated, highly weathered, jungle soils with a B horizon rich in sesquioxides or clays. Owing to the hot, humid climatic conditions under which

FIG. 4.4 Soil profile: spodosol (podsol). (Courtesy USDA Natural Resources Conservation Service.)

they exist, these soils are intensely active microbiologically and require constant replenishment of organic matter by the vegetation and from animal excretions and remains to stay fertile. The neutral to alkaline pH conditions of oxisols promote leaching of silicate and precipitation of iron and aluminum as sesquioxides. When oxisols are denuded of their arboreal vegetation, as in slash-and-burn agriculture, they quickly lose their fertility as a result of the intense microbial activity, which rapidly destroys soil organic matter. Because little organic matter is returned to the soil in its agricultural exploitation, conditions favor **lateriza-tion**, a process in which iron and aluminum oxides, silica, and carbonates are precipitated that cement the soil particles together and greatly reduce its porosity and water-holding capacity and make it generally unfavorable for plant growth.

Aridisols and **entisols** are desert soils that occur mostly in hot, arid climates at low latitudes. Aridisols have an ochreous surface soil and may show one or more subsurface horizons as follows: argillic horizon (a layer with silica and clay minerals dominating), cambic horizon (an altered, light-colored layer, low in organic matter, with carbonates usually present), natric horizon (dominant presence of sodium in exchangeable cation fraction), salic horizon (enriched in water-soluble salts), calcic horizon (secondarily enriched in $CaCO_3$),

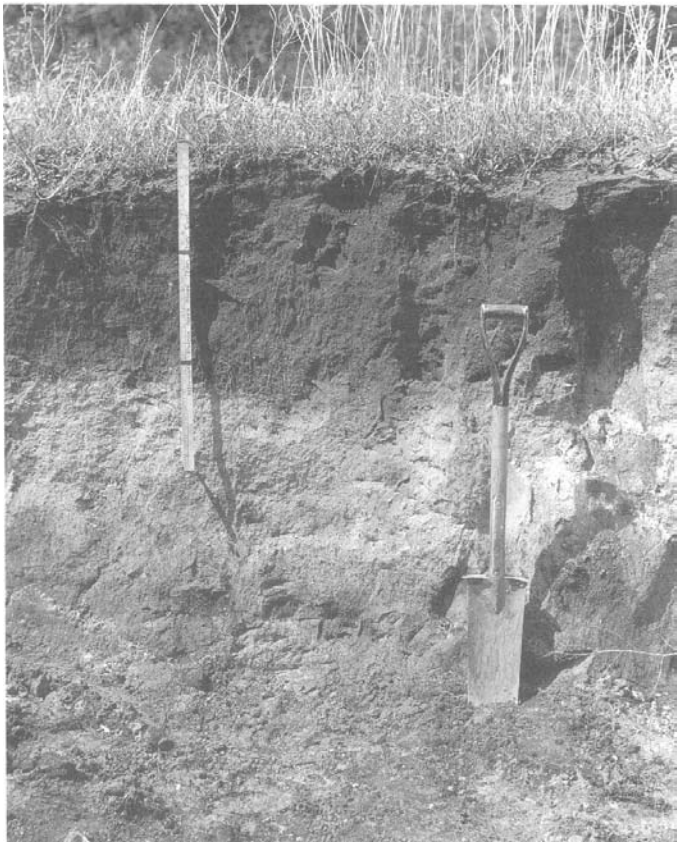


FIG. 4.5 Soil profile: mollisol (chernozem). (Courtesy USDA Natural Resources Conservation Service.)

gypsic horizon (secondarily enriched in $CaSO_4 \cdot 2H_2O$), and duripan horizon (primarily cemented by silica and secondarily by iron oxides and carbonates) (see Fuller, 1974; Buol et al., 1980). Entisols are poorly developed, immature desert soils without subsurface development. They may arise from recent alluvial deposits or from rock erosion (Fuller, 1974; Buol et al., 1980).

Desert soils are not fertile. It is primarily the lack of sufficient moisture that prevents the development of lush vegetation. However, insufficient nitrogen as a major nutrient and zinc, iron, and sometimes copper, molybdenum, or manganese as minor nutrients may also limit plant growth. Desert soils support a specially adapted macroflora and fauna that cope with the stressful conditions in such an environment. They also harbor a characteristic microflora of bacteria, fungi,

algae, and lichens. Actinomycetes, algae, and lichens may sometimes be dominant. Cyanobacteria seem to be more important in nitrogen fixation in desert soils than other bacteria. Desert soils can sometimes be converted to productive agricultural soils by irrigation. Such watering often results in extensive solubilization of salts from the subhorizons where they have accumulated during soil forming episodes. As a consequence, the salt level in the available water in the growth zone of the soil may increase to a concentration that becomes inhibitory to plant growth. The drainage water from such irrigated soil will also become increasingly salty and present a disposal and reuse problem.

Types of Microbes and Their Distribution in Mineral Soil

Microorganisms found in mineral soil include bacteria, fungi, protozoa, and algae and also viruses associated with these groups. A great variety of bacteria may be encountered. A portion of these may not yet have been cultured but are known to exist through analysis of DNA extracted from soil samples. This portion may significantly outnumber those that have been cultured. Morphological types of cultured bacteria include gram-positive rods and cocci, gram-negative rods and spirals, sheathed bacteria, stalked bacteria, mycelial bacteria (**actinomycetes**), budding bacteria, and others. In terms of oxygen requirements, they may be aerobic, facultative, or anaerobic. Physiological types include cellulolytic, pectinolytic, saccharolytic, proteolytic, ammonifying, nitrifying, denitrifying, nitrogen-fixing, sulfate-reducing, iron-oxidizing and -reducing, manganese-oxidizing and -reducing, and other types. Morphologically the dominant forms of culturable bacteria seem to be gram-positive cocci, probably representing the coccoid phase of *Arthrobacter* or possibly microaerophilic cocci related to *Mycococcus* (Casida, 1965). At one time, non-spore-forming rods were held to be the dominant form. Spore-forming rods are not very prevalent despite the fact that they are readily encountered when culturing soil in the laboratory. Numerical dominance of a given type does not necessarily speak for its biochemical importance in soil. Thus, nitrifying and nitrogen-fixing bacteria are less numerous than some others but of vital importance to the nitrogen cycle in soil. A given soil under a given set of conditions will harbor an optimum number of individuals of each resident bacterial group. These numbers will change with modification in prevailing physical and chemical conditions. Total viable counts in soils generally range from 10^5 g^{-1} in poor soil to 10^8 g^{-1} in garden soil.

The bacteria in soil are primarily responsible for mineralization of organic matter, for fixation of nitrogen, for nitrification, for denitrification (Campbell and Lees, 1967), and for some other geochemically important processes such as sulfate reduction, which cannot proceed in soil without the intervention of sulfate-

reducing bacteria. They play a significant role in mineral mobilization and immobilization. Some types of bacteria, especially **copiotrophs** (microorganisms requiring a nutrient-rich environment), often reside in microcolonies or biofilms on soil particles because it is there they find optimum nutrition and other requirements for their existence (see earlier section on Water Distribution in Mineral Soil). Conditions of nutrient supply, oxygen supply, moisture availability, pH, and E_h may vary widely from particle to particle, owing in part to the activity of different bacteria or other micro- or macroorganisms. Thus, soil contains many different microenvironments. The colonization of soil particles by bacteria may cause some particles to adhere to one another (Martin and Waksman, 1940, 1941), which means that bacteria can affect soil texture.

Fungi reside mainly in the O horizon and the upper A horizon of soil because they are, for the most part, strict aerobes and find their richest food supply in these sites (Atlas and Bartha, 1997). They are of great importance in the initial degradation of natural polymers such as cellulose and lignin, which are the chief constituents of wood and which most kinds of bacteria are unable to attack. They share the degradation products from these polymers with bacteria, which then mineralize them. Some fungi are predaceous and help control the protozoan population (Alexander, 1977, p. 67) and nematode populations (Pramer, 1964) in soil. Their mycelial growth habit causes them to grow over soil particles and penetrate the pore space of soil. They may also cause clumping of soil particles. The soil fungi include members of all the major groups: Phycomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes, and also slime molds. The last named are usually classified separately from fungi and protozoa, although they have attributes of both. In numbers, the fungi represent a much smaller fraction of the total microbial population than do the bacteria. Total numbers of fungi, expressed as propagules (spores, hyphae, hyphal fragments), may range from 10^4 to 10^6 per gram of soil.

Protozoa are also found in soil. They inhabit mainly the upper portion of soil where their food source (prey) is most abundant. They are represented by flagellates (Mastigophora), amoebae (Sarcodina), and Ciliates (Ciliata). Like fungi, they are less numerous than the bacteria, typically ranging from 7×10^3 to 4×10^5 per gram of soil (Alexander, 1977; Atlas and Bartha, 1997). The types and numbers of protozoa in a given soil depend on soil type and soil condition. Although both **saprozoic** and **holozoic** types occur, it is the latter that are of ecological importance in soil. Being predators, the holozoic forms help to keep the bacteria and, to a much lesser extent, other protozoa, fungi, and algae in check (Paul and Clark, 1996).

In the study of soils, cyanobacteria and algae are usually considered as a single group labeled algae, even though the cyanobacteria are prokaryotes and the algae are eukaryotes. Although both groups are associated mostly with aquatic environments, they do occur in significant numbers in the O horizon and

the uppermost portion of the A horizon in soils (Alexander, 1977; Atlas and Bartha, 1997) where sufficient light penetrates through translucent minerals and pore space. Overall, they are the least numerous of the various microbial groups, ranging from 10^2 to 10^4 per gram of soil just below the soil surface (Alexander, 1977), but as many as 10^6 g^{-1} have been reported (Atlas and Bartha, 1997). The most important true algal groups represented in soil are the green algae (chlorophytes) and diatoms (chrysophytes). Xanthophytes may also be present. Although cyanobacteria and algae are photosynthetic and therefore grow mostly at or just below the soil surface where light can penetrate, they may also be found below the light zone, where they seem to grow heterotrophically. Some cyanobacteria, green algae, and diatoms have been shown to be capable of heterotrophic growth in the dark. Some of the cyanobacteria in soil are capable of nitrogen fixation and may in some cases be more important in enriching soil in fixed nitrogen than other bacteria. The growth of algae in soil is dependent on adequate moisture and CO_2 supply. The latter is rarely limiting. The pH will influence which algae will predominate. Whereas cyanobacteria prefer neutral to alkaline soil, green algae will also grow in acid soil. When growing photosynthetically, cyanobacteria and algae are primary producers, generating at least some of the reduced carbon in soil on which heterotrophs depend. Because of their role as primary producers, cyanobacteria and algae are the pioneers in the formation of new soils, for instance in volcanic areas.

Bacterial distribution based on viable counts in mineral soils is illustrated in Table 4.3. In viable counts, the largest number of organisms occurs characteristically in the upper A horizon and the smallest in the B horizon. Aerobic

TABLE 4.3 Bacterial Densities at Different Depths of Different Soil Types on July 7, 1915

Soil depth (in.)	Bacterial densities (bacteria per gram of air-dried soil $\times 10^6$)			
	Garden soil	Orchard soil	Meadow soil	Forest soil
1	7.76	6.23	6.34	1.00
4	6.22	3.70	5.20	0.34
8	2.81	1.01	3.80	0.27
12	0.80	0.82	1.11	0.060
20	0.31	0.075	0.10	0.040
30	0.30	0.052	0.70	0.023

Source: Waksman (1916).

bacteria are generally more numerous than anaerobic bacteria, actinomycetes, fungi, or algae. In enumerations like those in Table 4.3, anaerobes decrease with depth to about the same degree as aerobes. This seems contradictory but may reflect the fact that most of the anaerobes that were enumerated by the methodology then in use were facultative.

Determination of microbial distribution in soil, when done by culturing as in the study summarized in Table 4.3, never yields an absolute estimate because no universal medium exists on which all living microbes can grow. A somewhat better estimate can be obtained through direct counts using fluorescence microscopy with soil preparations treated with special fluorescent reagents (see Chap. 7), provided viable cells can be distinguished from dead cells.

4.3 ORGANIC SOILS

In some special locations, **organic soils** or **histosols** are found. They form from rapid accumulation and slow decomposition of organic matter, especially plant matter, as a result of displacement of air by water, which prevents rapid and extensive microbial decomposition of the organic matter. These soils are thus sedimentary in origin and never the result of rock weathering. They consist of 20% or more organic matter (Lawton, 1955; Buol et al., 1989; Atlas and Bartha, 1997). Their formation is associated with the evolution of swamps, tidal marshes, bogs, and even shallow lakes. An organic soil such as peat may have an ash content of 2–50% and contain cellulose, hemicellulose, lignin and derivatives, heterogeneous complexes, fats, waxes, resins, and water-soluble substances such as polysaccharides, sugars, amino acids, and humins (Lawton, 1955). The pH of organic soils may range from 3 to 8.5. Examples of such soils are peat and "mucks." They accumulate to depths ranging from less than a meter to more than 8 m (Lawton, 1955) and are not stratified like mineral soils. They are rare in occurrence. Some are agriculturally very productive.

4.4 THE DEEP SUBSURFACE

In part because for around 100 years the study of microbial distribution in soil profiles had clearly shown microbes to decrease to negligible numbers with depth, the existence of microbial life below 50 m or more in the lithosphere was once considered rare at best. Recent investigations, however, have shown that living microbes exist in groundwater at depths as great as 3500 m (Szewzyk et al., 1994). Indeed, depending on the sampling site, bacterial population densities in

groundwater from depths of 50–3500 m can range from 10 to 10^8 mL^{-1} (Pedersen, 1993). Examples of locations of this groundwater in the deep subsurface are sediments and porous sedimentary rock strata (e.g., Sargent and Fliermans, 1989; Colwell et al., 1997) or cracked or fissured igneous rock, e.g., granite (Pedersen, 1993). Stratigraphically, bacteria are found only in permeable strata, which may be separated by over- and underlying impermeable strata (Sinclair and Ghiorse, 1989).

Besides inhabiting groundwater itself, bacteria in the deep subsurface may also live in microcolonies and biofilms on the surface of sediment particles and rock channels (Pedersen, 1993). To sample these sites, special drilling techniques have been developed to ensure that the organisms recovered are not contaminants introduced during the drilling and sampling process (Pederson, 1993; Griffin et al., 1997; Russell, 1997). Very diverse groups of culturable bacteria have been recovered, including Bacteria and Archaea, aerobes and anaerobes, mesophiles and thermophiles, autotrophs and heterotrophs, and representing a broad range of physiological types (Balkwill, 1989; Balkwill and Boone, 1997; Pedersen, 1993; Colwell et al., 1997; Kotelnikova and Pedersen, 1998). Fungi, protozoa, and algae have also been found, but mostly at shallower subsurface depths (Sinclair and Ghiorse, 1989).

The rate of microbial metabolism in the deep subsurface has been reported to be several orders of magnitude slower (Chapelle and Lovley, 1990; Phelps et al., 1994) than rates measured in soil. The very low in situ rates at specific deep subsurface sites were estimated by applying geochemical models. In one model, microbial CO_2 production from organic carbon along an aquifer flow path was estimated by taking into consideration CO_2 production from available organic matter, interaction of the produced CO_2 with carbonate in the aquifer matrix and the resultant changes in groundwater chemistry, the groundwater flow rate, and the length of the flowpath (Chapelle and Lovley, 1990). By comparison, direct application of small amounts of radiolabeled test substrates to subsurface samples from the same sites led to overestimation of in situ rates by two orders of magnitude or more. In dismissing metabolic rate measurements obtained by application of small amounts of test substrates as overestimations, the following needs to be remembered, however. Although the in situ nutrient supply in the deep subsurface is very limited, fresh supplies that stimulate metabolism may be introduced very episodically in cases where replenishment by groundwater movement is possible. Hence, metabolic rates in the subsurface may rise briefly at widely spaced time intervals. Because of the relatively short duration of these increased in situ rates, they have little influence on the average values obtained by application of geochemical models. Metabolic rate estimates obtained by addition of radiolabeled substrates to deep subsurface samples will give an indication of metabolic potential.

Much is still to be learned about the microbiology of the deep subsurface.

4.5 SUMMARY

The lithosphere of the Earth consists of rock, which may be igneous, metamorphic, or sedimentary. Rock is composed of intergrown minerals. The rock surface and, in the case of porous rock, its interior may be habitats for microbes. Rock may be broken down by weathering, which may ultimately lead to formation of mineral soil. Some of the rock minerals become chemically altered in the process. Weathering may be biological, especially microbiological, as well as chemical and physical.

Progress of mineral soil development is recognizable in a soil profile. A vertical section through mineral soil may reveal more or less well developed horizons. Typical horizons of spodosols and mollisols include the litter zone (O horizon), a leached layer (A horizon), an enriched layer (B horizon), and the parent material (C horizon). The aspect of these horizons varies with soil type. Climate is one of several important determinants of soil type. The horizons are the result of intense biological activity in the litter zone and A horizon. Much of the organic matter in the litter zone is microbially solubilized and at least partly degraded. Soluble components are washed into the A horizon or transported there by some invertebrates, where they may be further metabolized and where they contribute directly or indirectly to transformation of some of the mineral matter. Soluble products, especially inorganic ones, formed in the A horizon may be washed into the B horizon. The more refractory organic matter in the soil accumulates as humus, which contributes to the soil's texture, water-holding capacity, and general fertility. Mineral soil may be 50% solid matter and 50% pore space. The pore space is occupied by gases such as N_2, CO_2, and O_2 and by water. Water also surrounds soil particles to varying degrees. Microbes, including bacteria, fungi, protozoa, and algae, may inhabit the soil pores or live on the surface of soil particles. They are most numerous in the upper layer of soil.

Not all soils can be classified as mineral soils. A few are organic and have a different origin. They arise from the slow decomposition of organic matter, mainly plant residues, which accumulates by sedimentation, as in swamps, marshes, and shallow lakes. They are not stratified and usually have low mineral content.

Soil is not the only important microbial habitat of the lithosphere. Microbes have now been detected in the deep subsurface of the lithosphere, at depths as great as 3500 m. Although bacteria, fungi, protozoa, and algae can be found at shallower depths, bacteria predominate in deep zones. The organisms can be found in permeable strata formed by sediments, sedimentary rock, and cracked or fissured igneous rock. They inhabit the pore water in these strata and also the exposed sediment particle or rock surfaces, on which they may form microcolonies or biofilms.

The bacteria exhibit great diversity morphologically and physiologically. Their in situ metabolic rates appear to very low owing to nutrient limitation. Much remains to be discovered about life in the deep subsurface of the lithosphere.

REFERENCES

Alexander M. 1977. Introduction to Soil Microbiology. 2nd ed. New York: Wiley.

Atlas RM, Bartha R. 1997. Microbial Ecology. Fundamentals and Applications. 4th ed. Menlo Park, CA: Addison Wesley Longman.

Balkwill DL. 1989. Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. Geomicrobiol J 7:33–52.

Balkwill, DL, Boone DR. 1997. Identity and diversity of microorganisms cultured from subsurface environments. In: Amy PS, Haldeman DL, eds. The Microbiology of the Terrestrial Deep Subsurface. Boca Raton, FL: CRC Lewis, pp 105–117.

Barker WW, Banfield JF. 1996. Biologically versus inorganically mediated weathering reactions: Relationships between minerals and extracellular microbial polymers in lithobiontic communities. Chem Geol 132:55–69.

Barker WW, Banfield JR. 1998. Zones of chemical and physical interaction at interfaces between microbial communities and minerals: A model. Geomicrobiol J 15:223–244.

Bennett PC, Hiebert FK, Choi WJ. 1996. Microbial colonization and weathering of silicates in a petroleum-contaminated groundwater. Chem Geol 132:45–53.

Berner RA, Holdren GR Jr. 1977. Mechanism of feldspar weathering: Some observational evidence. Geology 5:369–372.

Berthelin J. 1977. Quelques aspects des mécanismes de transformation des minéraux des sols par les micro-organismes hétérotrophes. Sci Bull AFES 1:13–24.

Berthelin J, Boymond D. 1978. Some aspects of the role of heterotrophic microorganisms in the degradation of minerals in waterlogged soils. In: Krumbein WE, ed. Environmental Biogeochemistry and Geomicrobiology. Ann Arbor, MI: Ann Arbor Sci, pp 659–673.

Brady PV, Carroll SA. 1994. Direct effects of CO_2 and temperature on silicate weathering: Possible implications for climate control. Geochim Cosmochim Acta 58:1853–1856.

Brock TD. 1975. Effect of water potential on growth and iron oxidation by *Thiobacillus ferrooxidans*. Appl Microbiol 29:495–501.

Brock TD, Smith DW, Madigan MT. 1984. Biology of Microorganisms. 4th ed. Englewood Cliffs, NJ: Prentice-Hall.

Brown AD. 1976. Microbial water stress. Bacteriol Rev 40:803–846.

Browne BA, Driscoll CT. 1992. Soluble aluminum silicates: Stoichiometry, stability, and implications for environmental geochemistry. Science 256:1667–1670.

Bunting BT. 1967. The Geography of Soil. London: Hutchison Univ Library.

Buol SW, Hole FD, McCracken RJ. 1980. Soil Genesis and Classification. 2nd ed. Ames, IA: Iowa State Univ Press.

Buol SW, Hole FD, McCracken RJ. 1989. Soil Genesis and Classification. 3rd ed. Ames, IA: Iowa State Univ Press.

Campbell NER, Lees H. 1967. The nitrogen cycle. In: McLaren AD, Petersen GH, eds. Soil Biochemistry. Vol 1. New York: Marcel Dekker, pp 194–215.

Casida EL. 1965. Abundant microorganisms in soil. Appl Microbiol 13:327–334.

Chapelle FH, Lovley DR. 1990. Rates of microbial metabolism in deep coastal plain aquifers. Appl Environ Microbiol 56:1865–1874.

Chou L, Wollast R. 1984. Study of the weathering of albite at room temperature and pressure with a fluidized bed reactor. Geochim Cosmochim Acta 48:2205–2218.

Colwell FS, Onstott TC, Delwiche ME, Chandler D, Fredrickson JK, Yao Q-J, McKinley JP, Boone DR, Griffiths R, Phelps TJ, Ringelberg D, White DC, LaFreniere L, Balkwill D, Lehman RM, Konisky J, Long PE. 1997. Microorganisms from deep, high temperature sandstones: Constraints on microbial colonization. FEMS Microbiol Rev 20:425–435.

Costerton JW, Lewandowski Z, deBeer D, Caldwell D, Korber D, James G. 1994. Biofilms, the customized microniche. J Bacteriol 176:2137–2142.

Dommergues Y, Mangenot F. 1970. Ecologie Microbienne du Sol. Paris: Masson.

Ehrlich HL. 2001. Interactions between microorganisms and minerals under anaerobic conditions. In: Huang PM, ed. Interactions Between Soil Particles and Microorganisms and Their Impact on the Terrestrial Environment. IUPAC Ser. New York: Wiley, Chap. 11 (in press).

Friedman, EI. 1982. Endolithic microorganisms in the Antarctic cold desert. Science 215:1045–1053.

Fuller WH. 1974. Desert soils. In: Brown GW, ed. Desert Biology. Vol II. New York: Academic Press, pp 31–101.

Golubic S, Schneider J. 1979. Carbonate dissolution. In: Trudinger PA, Swaine DJ, eds. Biogeochemical Cycling of Mineral Forming Elements. Amsterdam: Elsevier, pp 107–129.

Golubic S, Perkins DD, Lukas KJ. 1975. Boring microorganisms and microborings in carbonate substrates. In: Frey RW, ed. The Study of Trace Fossils. New York: Springer-Verlag, pp 229–259.

Griffin WT, Phelps TJ, Colwell FS, Fredrickson JK. 1997. Methods for obtaining deep subsurface microbiological samples by drilling. In: Amy PS, Haldeman DL, eds. The Microbiology of the Terrestrial Deep Subsurface. Boca Raton, FL: CRC Lewis, pp. 23–44.

Harris RF, Gardner WR, Adebayo AA, Sommers LE. 1970. Agar dish isopiestic equilibration method for controlling the water potential of solid substrates. Appl Microbiol 19:536–537.

Hiebert FK, Bennett PC. 1992. Microbial control of silicate weathering in organic-rich groundwater. Science 258:278–281.

Jones CG, Shachak M. 1990. Fertilization of the desert soil by rock-eating snails. Nature (Lond) 346:839–841.

Jones D, Wilson MJ, McHardy WJ. 1981. Lichen weathering of rock-forming minerals: Application of scanning electron microscopy and microprobe analysis. J Microsc 124:95–104.

Kotelnikova S, Pedersen K. 1998. Distribution and activity of methanogens and homoacetogens in deep granitic aquifers at Äspö Hard Rock Laboratory, Sweden. FEMS Microbiol Ecol 26:121–134.

Kuznetsov SI, Ivanov MV, Lyalikova NN. 1963. Introduction to Geological Microbiology. English trans. New York: McGraw-Hill.

Lang ARG. 1967. Osmotic coefficients and water potentials of chloride solutions from 0–40°C. Aust J Chem 20:2017–2023.

Lawton K. 1955. Chemical composition of soils. In: Bear FE, ed. Chemistry of the Soil. New York: Van Nostrand Reinhold, pp 53–84.

Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC. 1996. Humic substances as electron acceptors for microbial respiration. Nature (Lond) 382:445–448.

Lucas Y, Luizao FJ, Chauvel A, Rouiller J, Nahon D. 1993. The relation between biological activity of the rain forest and mineral composition of soils. Science 260:521–523.

Martin JP, Waksman SA. 1940. Influence of microorganisms on soil aggregation and erosion. Soil Sci 50:29–47.

Martin JP, Waksman SA. 1941. Influence of microorganisms on soil aggregation and erosion. II. Soil Sci 52:381–394.

Oelkers EH, Schott J, Devidal J-L. 1994. The effect of aluminum, pH, and chemical affinity on the rates of aluminosilicate dissolution reactions. Geochim Cosmochim Acta 58:2011–2024.

Paul EA, Clark FE. 1996. Soil Microbiology and Biochemistry. 2nd ed., San Diego: Academic Press.

Pedersen K. 1993. The deep subterranean biosphere. Earth-Sci Rev 34:243–260.

Phelps TJ, Murphy EM, Pfiffner SM, White DC. 1994. Comparison between geochemical and biological estimates of subsurface microbial activities. Microb Ecol 28:335–349.

Pramer D. 1964. Nematode trapping fungi. Science 144:382–388.

Russell CE. 1997. The collection of subsurface samples by mining. In: Amy PS, Haldeman DL, eds. The Microbiology of the Terrestrial Deep Subsurface. Boca Raton, FL: CRC Lewis, pp 45–59.

Sargent KA, Fliermans CB. 1989. Geology and hydrology of the deep subsurface microbiology sampling sites at the Savannah River Plant, South Carolina. Geomicrobiol J 7:3–13.

Shachak M, Jones CG, Granot Y. 1987. Herbivory in rocks and the weathering of a desert. Science 236:1098–1099.

Sinclair JL, Ghiorse WC. 1989. Distributrion of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol J7:15–31.

Stevenson FJ. 1994. Humus Chemistry. Genesis, Composition, Reactions. New York: Wiley.

Szewzyk U, Szewzyk R, Stenström T-A. 1994. Thermophilic, anaerobic bacteria isolated from a deep borehole in granite in Sweden. Proc Natl Acad Sci (USA) 91:1810–1813.

Ullman WJ, Kirchman DL, Welch SA, Vandevivere P. 1996. Laboratory evidence for microbially mediated silicate mineral dissolution in nature. Chem Geol 132:11–17.

Waksman SA. 1916. Bacterial numbers in soils at different depths and in different seasons of the year. Soil Sci 1:363–380.

Waksman SA, Starkey RL. 1931. The Soil and the Microbe. New York: Wiley.
Welch SA, Ullman WJ. 1993. The effect of organic acids on plagioclase dissolution rates and stoichiometry. Geochim Cosmochim Acta 57:2725–2736.
Welch SA, Vandevivere P. 1995. Effect of microbial and other naturally occurring polymers in mineral dissolution. Geomicrobiol J 12:227–238.
Wieland E, Stumm W. 1992. Dissolution kinetics of kaolinite in acidic aqueous solutions at 25°C. Geochim Cosmochim Acta 56:3339–3355.

5

The Hydrosphere as a Microbial Habitat

5.1 THE OCEANS

Physical Attributes

The oceans are a habitat for various forms of life, ranging from the largest organism anywhere on Earth to the smallest. The **fauna** includes various vertebrates (mammals, birds, reptiles, fish) as well as a wide range of invertebrates. The **flora** includes the algae—from the macroscopic kelps to the small unicellular forms. The **plankton** includes the floating biota. The **phytoplankton** includes free-floating algae such as diatoms and dinoflagellates, and the **zooplankton** includes the free-floating microscopic invertebrates and protozoa. The **bacterioplankton** consists of free, unattached bacterial forms that include some kinds of cyanobacteria. In considering the ecology of the oceans, cyanobacteria, which are prokaryotes, have often been considered with the true algae, which are eukaryotes.

The oceans cover about 70% of the Earth's surface, occupying an area of 3.6×10^8 km^2 and a volume of 1.37×10^9 km^3, which amounts to 1.41×10^{21} kg of water. By comparison, the total mass of the Earth is estimated to be 5.98×10^{24} kg. Because of the unequal distribution of the continents between the northern and southern hemispheres of the present Earth, only 60.7% of the northern hemisphere is covered by oceans, whereas 80.9% of the southern

hemisphere is covered by them. The world's major oceans include the Atlantic Ocean (16.2% of the Earth's surface), the Pacific Ocean (32.4% of the Earth's surface), the Indian Ocean (14.4% of the Earth's surface), and the Arctic Ocean (2.8% of the Earth's surface). The average depth of all oceans is 3795 m. The average depth of the Atlantic Ocean 3296 m, that of the Pacific Ocean is 4282 m, that of the Indian Ocean is 3693 m, and that of the Arctic Ocean is 1205 m. The greatest depth in the oceans occurs in **ocean trenches**. For instance, in the Pacific Ocean, the water depth of the Marianas Trench is 10,500 m. In the Atlantic Ocean, the Puerto Rico Trench has a water depth of 8650 m, and in the Indian Ocean, the Java Trench has a water depth of 7450 m. Shallow ocean depths are encountered in the marginal seas along the coasts of the continents. These seas are usually less than 2000 m and frequently less than 1000 m deep. Figure 5.1 shows the oceans of the world (e.g., Williams, 1962; Bowden, 1975).

An ocean includes a basin that is surrounded by a continental margin with several structural features. Projecting from each continental shore is the **continental shelf**, all shelves together encompassing about 7.5% of the ocean area. Each shelf slopes gently downward in the direction of the ocean at an average angle of 7 min to a water depth of about 130 m. Its average width is about 65 km, but its width may range from 0 to 1290 km, the greatest distance being represented by the shelf projecting from the coast of Siberia into the North Polar Sea. The waters over the continental shelves are a biologically important part of the oceans. They are the site of high biological productivity because they receive a significant contribution of nutrients in general runoff from the adjacent land, particularly from rivers emptying into the waters over the shelf area.

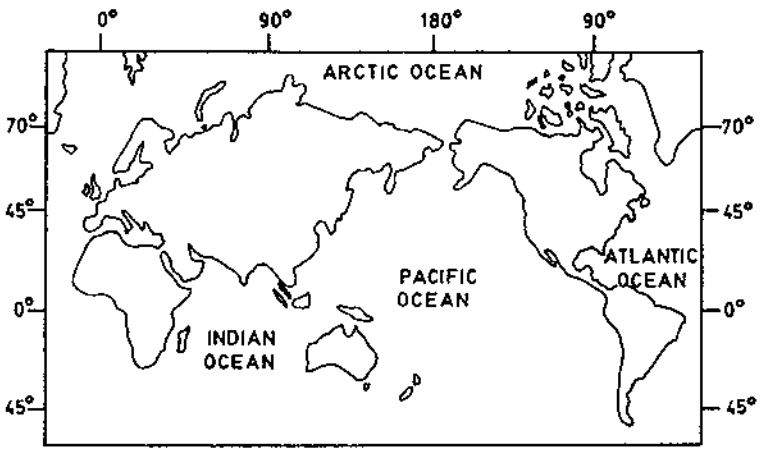


FIG. 5.1 Oceans of the world.

At the edge of the continental shelf, the ocean floor drops sharply at an average angle of 4° (range $1-10^0$) to abyssal depths of about 3000 m. This is the region of the **continental slope** and constitutes about 12% of the ocean area. In some places the slope is cut by deep canyons. They often occur at the mouths of large rivers (e.g., Hudson River, Amazon River). Many canyons were produced by **turbidity currents** over geologic time. Such currents consist of strong water movements that carry a high sediment load picked up as a river flows oceanward. As the river meets the sea, the sediment is dropped, and as it settles it abrades the slope. Marine canyons may also be cut by slumping of an unstable sediment deposit on a portion of the continental slope and the consequent abrasion of the slope. On occasion, the continental slope may be interrupted by a terraced region, as in the case of Blake Plateau off the southern Atlantic coast of the United States. This particular shelf is about 302 km wide and drops gradually from a depth of 732 m to one of 1100 m over this distance. It was gouged out of the continental slope by the northward flowing Gulf Stream.

At the foot of the slope lies the **continental rise**, consisting of accumulations of sediment carried downslope by turbidity currents. Such deposits may extend for 100 km or more from the foot of the continental slope. The continental rise may form fanlike structures in some places and wedges in others. An idealized profile of a continental margin is shown in Figure 5.2.

The **ocean basin** takes up 80% of the ocean area. Its floor, far from being a flat expanse, as some once believed, often exhibits a rugged topography. Submarine mountain ranges cut by fracture zones and rift valleys stretch over thousands of kilometers as the mid-ocean ridge systems where new ocean floor is created (see Chap. 2). Elsewhere, somewhat more isolated submarine mountains, some of which are active and others dormant volcanoes, dot the ocean floor. Some of the seamounts have flattened tops and have been given the special name of **guyots**. Some of the flattened tops of seamounts, especially in the Pacific Ocean, reach surface waters at depths of 50–100 m where the temperature is about 21°C. In these positions, the flat tops may serve as substratum for colonization by corals (coelenterates) and coralline algae, which then form atolls and reefs.

Covering the ocean floor almost everywhere are **sediments**. They range in thickness from 0 to 4 km, with an average thickness of 300 m. Their rate of accumulation varies, being slowest in mid-ocean (less than 1 cm per 10^3 years) and fastest on continental shelves (10 cm per 10^3 years). These rates may be even greater in some inland seas and gulfs (e.g., 1 cm per 10–15 years in the Gulf of California and 1 cm per 50 years in the Black Sea). In some regions of the deep ocean, the sediments consist mainly of deposits of siliceous and/or calcareous remains of marine organisms. The siliceous remains are derived from the frustules of diatoms (algae) and the support skeleton and spines of radiolarians (protozoa). The calcareous remains are derived from the tests of foraminifera (protozoa), carbonate platelets from the walls of coccolithophores (algae), and

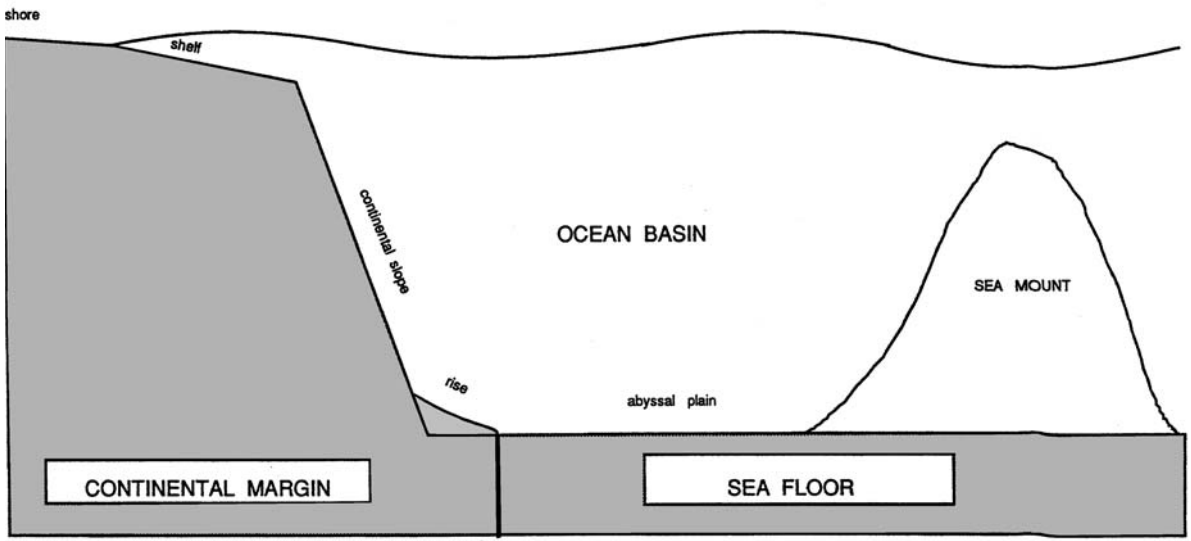


FIG. 5.2 Schematic representation of a profile of an ocean basin.

shells from pteropods (mollusks). **Diatomaceous oozes** predominate in colder waters [e.g., in the North Pacific between 40° and 70° north latitude and 140° west to 145° east longitude, according to Horn et al., (1972)]. **Radiolarian oozes** predominate in warmer waters [e.g., in the North Pacific between 5° and 20° north latitude and 90° and 180° west longitude, according to Horn et al. (1972)]. **Calcareous oozes** are found mainly in warmer waters on ocean bottoms no deeper than 4550–5000 m (e.g., in the North Pacific between 0° and 10° north latitude and 80° and 180° west longitude (Horn et al., 1972). At greater depths, the CO_2 concentration in the water is high enough to cause dissolution of carbonate unless the structures are enclosed in a protective membrane.

Other vast areas of the ocean floor are covered by clays (*red clays* or *brown mud*), which are probably of terrigenous origin and washed into the sea by rivers and general runoff from continents and islands and carried into the ocean basins by ocean currents, mudflows, and turbidity currents. At high latitudes in both hemispheres, particularly on and near continental shelves, ice-rafted sediments are found. They were dropped into the ocean by melting icebergs that had previously separated from glacier fronts that had picked up terrigenous debris during glacial progression. Except for ice-rafted detritus, only the fine portion of terrigenous debris (clays and fine silts) is carried out to sea. The clay particles are defined as less than 0.004 mm in diameter, and the silt particles are defined as being in a size range of 0.004–0.1 mm in diameter. Figure 5.3 shows the appearance of some Pacific Ocean sediments under the microscope.

The Ocean in Motion

A significant portion of the ocean is in motion at all times (e.g., Williams, 1962; Bowden, 1975). The causes of this motion are (1) wind stress on surface waters, (2) Coriolis force arising from the rotation of the Earth, (3) density variation of seawater resulting from temperature and salinity changes, and (4) tidal movement due to gravitational influences on the water exerted by the sun and moon. Surface currents (Fig. 5.4) are prominent in regions of prevailing winds, such as the trade winds, which blow from east to west at about 20° north and south latitudes; the westerlies, which blow from west to east between 40° and 60° north and south latitude; and the easterly polar winds, which blow in a westerly direction south of the Arctic Ocean. The effect of these winds, together with the deflecting influence of the continents and the Coriolis force, is to set up surface circulations in the form of gyrals between the north and south poles in each major ocean. They are the North Subtropical Gyral (large), the North Tropical Gyral (small), South Tropical Gyral (small), South Subtropical Gyral (large); and the Antarctic current, which circulates around Antarctica from west to east (Fig. 5.4A). Thus, the Gulf Stream, together with the Canary Current and the North Equatorial Current, is part of the North Subtropical Gyral of the Atlantic Ocean (Fig. 5.4B). The flow

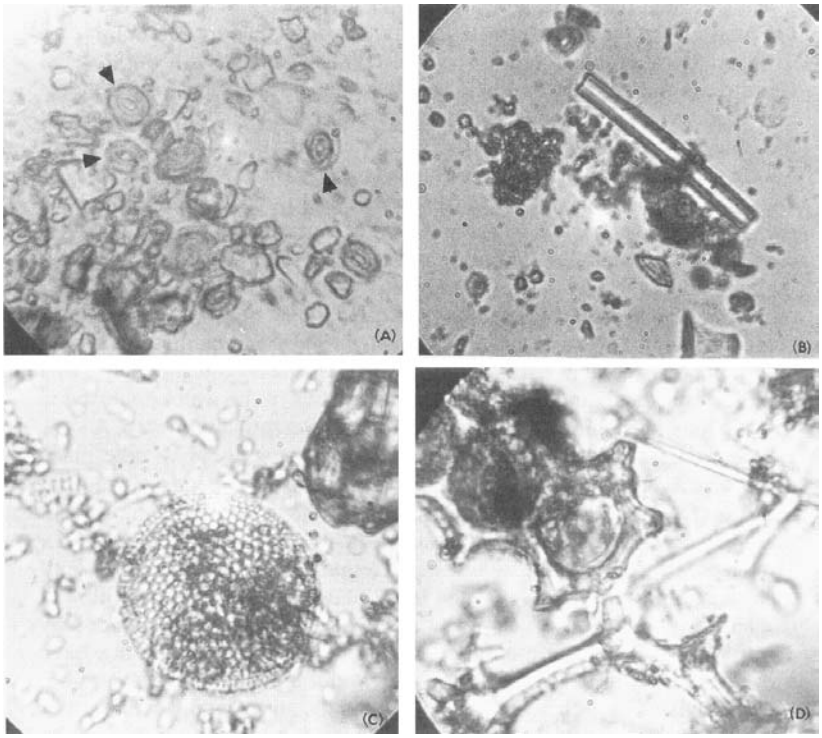


FIG. 5.3 Microscopic appearance of marine sediments (×1750). (A) Atlantic sediment showing coccoliths ($CaCO_3$) (arrows) and clay particles. (B) Atlantic sediment showing diatom frustules (SiO_2) and other debris. (C) Pacific sediment showing a centric diatom frustule (SiO_2) and other debris. (D) Pacific sediment showing fragments of radiolarian tests (SiO_2).

rates of the waters in these gyrals and segments of them vary. The flow rate of the water in the Gulf Stream is fastest for any surface current, 250 cm sec^{-1}. Other currents have flow rates that fall mostly in the range of 25–65 cm sec^{-1}.

Meanders in the Gulf Stream in the Atlantic and the Kuroshio Current in the Pacific Ocean may give rise to so-called rings, small closed current systems that may measure as much as 300 km in diameter and may have a depth as great as 2 km. Such rings may move 5–10 km per day. The chemical, physical, and biological characteristics of the water enclosed in a ring may be significantly different from those of the surrounding water. A slow exchange of solutes and biota as well as heat transfer may take place across the boundary of a ring. Rings thus constitute means of nutrient transport from ocean currents. The rings may ultimately rejoin the current that spawned them (Gross, 1982; Richardson, 1993;

Ring Group, 1981). More recently **anticyclones** have been reported to arise from the Gulf Stream in addition to the rings, and **meddies** north of the Strait of Gibraltar (Richardson, 1993). Whereas rings have a cold water core surrounded by a warm water layer and a counterclockwise rotation, anticyclones have a warm water core surrounded by colder water and clockwise rotation. Meddies have a core that is more saline than the surrounding ocean water and a clockwise rotation. Collectively these formations are known as **ocean eddies**.

Deep water is also in motion. Its movement appears to be caused by slow diffusion resulting from density differences of water masses through broad zones in the oceans. Some deep currents that have been measured in the Atlantic Ocean have a velocity between 1 and 2 cm sec^{-1} (Dietrich and Kalle, 1965, pp. 399, 407; Gross, 1982). Occasional short bursts in velocity may occur. The bottom current movement is influenced by bottom topography.

Deep water may rise toward the surface in a process called **upwelling**. This results from the moving apart of two surface water masses, causing deep water to rise to take the place of the divergent waters. The moving apart of the water masses is called a **divergence** (Williams, 1962). Upwelling of deep water may also result when winds blow large surface water masses away from coastal regions (Smith, 1968). Deep water is relatively rich in mineral nutrients, including

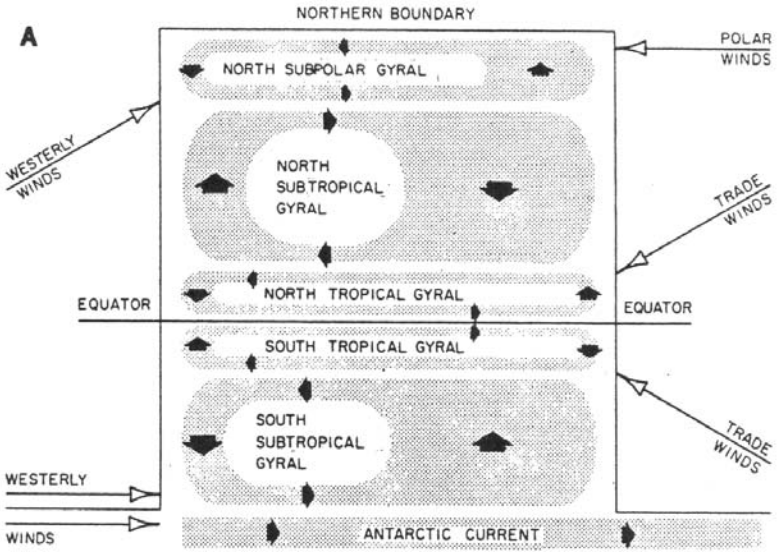


FIG. 5.4 Oceanic surface currents. (A) Schematic representation of the prevailing winds and their effects on the surface currents of an imaginary rectangular ocean. (*continued on next page*)

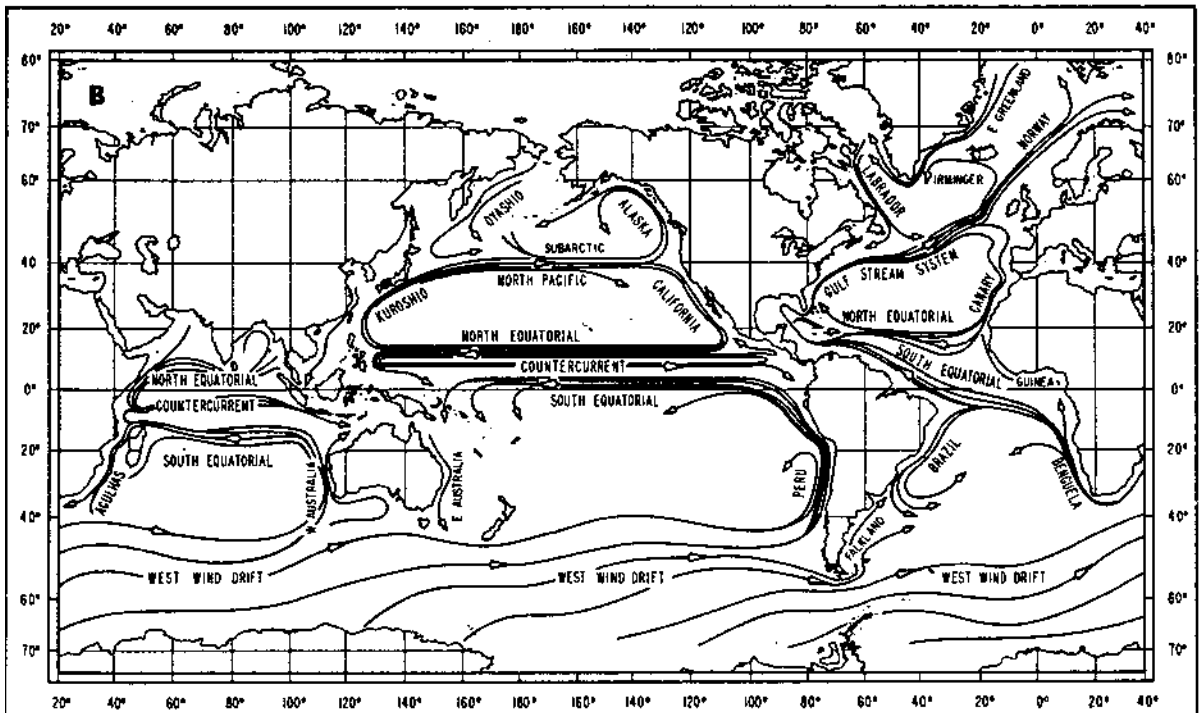


FIG. 5.4 (*continued*) Oceanic surface currents. (B) Average surface currents of the world's oceans. (From Williams, 1962, with permission.)

TABLE 5.1 Some Constituents of Seawater ($\mu g\ L^{-1}$)

Major constituents		Minor constituents		Minor constituents	
Cl	1.9×10^7	Si	3×10^3	Cu	3
Na	1.1×10^7	N	6.7×10^2	Fe	3
Mg	1.3×10^6	Li	1.7×10^2	U	3
S (SO_4)	9.0×10^5	Rb	1.2×10^2	As	2.6
Ca	4.1×10^5	P	90	Mn	2
K	3.9×10^5	I	60	Al	1
Br	6.7×10^4	Ba	20	Co	0.4
C (CO_3, HCO_3)	2.8×10^4	Mo	10	Se	9×10^{-2}
B	4.5×10^3	Zn	10	Pb	3×10^{-2}
		Ni	7	Ra	1×10^{-7}

Source: Values taken from *Marine Chemistry* (1971).

nitrate and phosphate, and thus upwelling is of great ecological consequence because it replenishes biologically depleted nutrients in the surface waters. Regions of upwelling are therefore very fertile. An important region of upwelling in the eastern Pacific Ocean occurs off the coast of Peru. A disturbance in the surface water circulation in the southern Pacific can result in failure of upwelling in this region (El Niño) and can spell disaster for the fisheries of the area.

Where a denser surface water mass meets a lighter water mass, a **convergence** occurs, and the heavier water will sink to a level where it meets water of its own density. This phenomenon is important because the denser, sinking surface water will carry oxygen to the deep waters. Important convergences in the oceans occur at high latitude in both hemispheres.

Chemical and Physical Properties of Seawater

Seawater is saline. Some important chemical components of seawater, listed in decreasing order of concentration, are presented in Table 5.1 (see also Marine Chemistry, 1971). Of these components, chloride (55.2%), sodium (30.4%), sulfate (7.7%), magnesium (3.7%), calcium (1.16%), potassium (1.1%), bromide (0.1%), strontium (0.04%), and borate (0.07%) account for 99.5% of the total salts in solution. Because these components generally occur in constant proportions relative to each other in true ocean waters, it has been possible to estimate salt concentrations in seawater samples by merely measuring chloride concentration. The chloride concentration in grams per kilogram (chlorinity, Cl) is related to the total salt concentration in grams per kilogram (salinity, S) by the relationship*

$$S\ (‰) = 0.030 + 1.8050Cl\ (‰) \tag{5.1}$$

*The symbol ‰ represents parts per thousand or grams per kilograms.

The salinity so determined is an estimate of the total amount of solid material in a unit mass of seawater in which all carbonate salts have been converted to oxides and all bromide and iodide has been replaced by chloride, and in which all organic matter has been completely oxidized. For reference purposes, the salinity of standard seawater has been taken as 34.3‰. The actual salinity of different parts of the world oceans can vary from less than 34‰ to almost 36‰ (Dietrich and Kalle, 1965, p. 156).

Table 5.2 lists the salinities of some different marine waters as well as those of some saline lakes. It must be pointed out that whereas the Great Salt Lake in Utah has a salt composition that is qualitatively similar to that of oceans, some other inland hypersaline water bodies, such as the Dead Sea at the mouth of the Jordan River, have a different salt composition. In the Dead Sea the dominant cations are in descending order, Mg ($\sim$44 g L^{-1}), Na (40 g L^{-1}), Ca (17 g L^{-1}), and K (7.5 g L^{-1}), and the dominant cations are Cl (225 g L^{-1}) and Br (5.5 g L^{-1}) (Nissenbaum, 1979).

Although some portions of the salts in seawater derive from the runoff from the continents and the weathering of minerals in the surficial sediments, a most important contribution to seawater solutes is made by hydrothermal discharges from vents at the mid-ocean spreading centers. These discharges are the consequence of seawater penetration deep into the porous basalt (up to 1–3 km depths) at the ocean floor, where the seawater then reacts with constituents of the basalt in various ways. The reactions include the reduction of seawater sulfate to hydrogen sulfide by ferrous iron in the basalt. They also include the removal of magnesium from seawater as magnesium hydroxide and the incorporation of seawater calcium into minerals such as plagioclase to form new aluminosilicate minerals such as clinozoisite or Ca-rich amphibole, accompanied by the generation of acidity. In the case of Ca incorporation into plagioclase, this can be illustrated by the reaction

$$3CaAl_2Si_2O_8 + Ca^{2+} + 2H_2O \rightarrow 2Ca_2Al_3Si_3O_{12}(OH) + 2H^+ \qquad (5.2)$$

TABLE 5.2 Salinities (‰) of Some Marine Waters and Salt Lakes

Gulf of Bothnia	2–6	(Smith, 1974)
Baltic Sea	6–17	(Smith, 1974)
Black Sea	16–18	(Smith, 1974)
Mediterranean Sea	37–39	(Bowden, 1975)
Red Sea	40–41	(Bowden, 1975)
Dead Sea	320	(ZoBell, 1946)
Great Salt Lake	320	(Zobell, 1946)
Ocean bottoms	34.66–34.92	(Defant, 1961)

The resultant acidity is the cause of leaching of some other components from the basalt such as hydrogen sulfide from pyrrhotite and base metals from some other basalt minerals. All these reactions are possible because of high hydrostatic pressure exerted on the solution in the basalt at these depths and because of the high temperature (~350°C) from heat diffusion from underlying magma chambers into the reaction zone of the basalt. The resultant chemically altered seawater is forced upward by hydrostatic pressure through porous channels and fissures in the basalt. It is ultimately discharged as hydrothermal solution from vents at the spreading centers into the overlying seawater and mixed with it (Bischoff and Rosenbauer, 1983; Edmond et al., 1982; Seyfried and Janecky, 1985; Shanks et al., 1981) (see also Chaps. 2, 16 and 19). These reactions contribute significantly to the stability of seawater composition.

Seawater contains a pH buffering system that consists of bicarbonate and carbonate ions, borates, and silicates. The carbonate plus bicarbonate ions constitute 0.35% of the solutes in seawater. Together, these buffers keep the pH of seawater in the range of 7.5–8.5. Surface seawater pH tends to fall into a narrow range of 8.0–8.5. At depth, seawater pH may approach 7.5. To a major extent, the variation in pH of seawater with depth may be related to oxygen utilization in respiration by marine organisms, which results in CO_2 production from organic carbon. To a lesser extent, it may be related to carbonate mineral (e.g., $CaCO_3$) dissolution (Park, 1968). Figure 5.5 illustrates pH variation with depth at one particular station in the Pacific Ocean.

Because of the alkaline pH and elevated E_h of seawater, nutritionally available iron appears to be a limiting micronutrient for bacterioplankton and

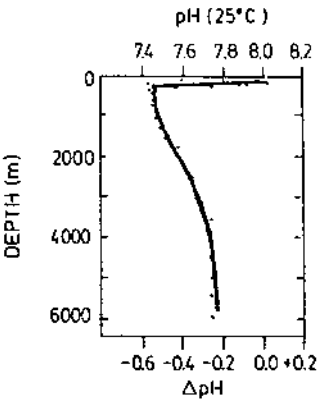


FIG. 5.5 Vertical profile of pH at station 54°46′N, 138°36′W in the northeastern Pacific Ocean. (Adapted with permission from PK Park, Seawater hydrogen-ion concentration: vertical distribution, Science 162: 357–358. Copyright 1968 by the American Association for the Advancement of Science.)

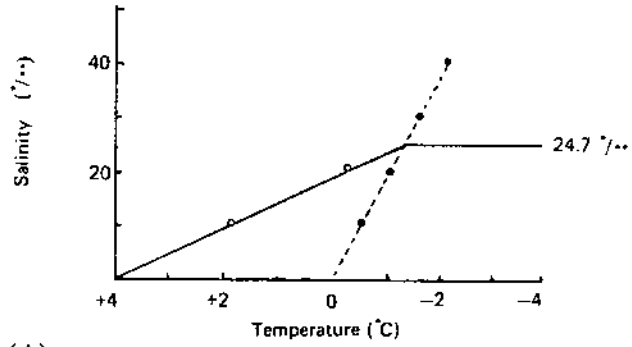
phytoplankton (Tortell et al., 1999; Hutchins et al., 1999; Gelder, 1999). This is because the iron under these conditions will be ferric and, unless complexed, will predominate in the form of insoluble hydroxide, oxyhydroxides, and oxides. Various bacteria and algae release ligands (siderophores) that complex ferric iron and thus keep it in solution and make it nutritionally available (Tortell et al., 1999; Hutchins et al., 1999; Martinez et al., 2000). Growth by some non-siderophore-producing marine bacteria in iron-limited waters can be stimulated by exogenous siderophores (Guan et al., 2001). In at least some parts of the oceans, it is possible to stimulate the growth of bacterioplankton and phytoplankton by fertilizing ocean water with iron (Coale et al., 1996; Church et al., 2000).

The salts dissolved in seawater impart a special osmotic property to it. The *osmotic pressure* of seawater is of the order of magnitude of the internal pressure of bacterial cells or the cell sap of eukaryotic cells. At a salinity of 35‰ and a temperature of 0°C, seawater has an osmotic pressure of 23.37 bar (23.07 atm), whereas at the same salinity but at 20°C it has an osmotic pressure of 25.01 bar (24.69 atm). Clearly, then, the osmotic pressure of seawater is not deleterious to living cells.

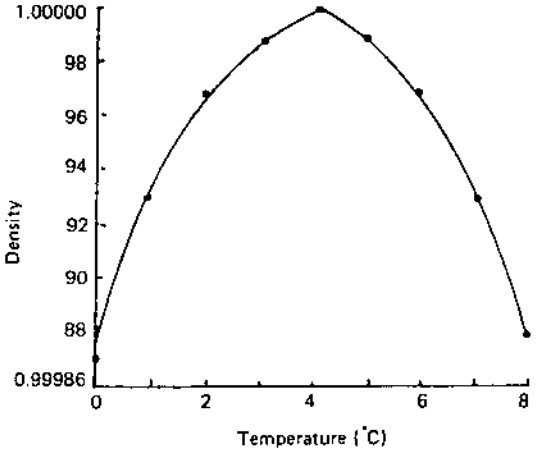
With increasing depth in the water column, hydrostatic pressure becomes a significant factor in the life of microbes and other forms of life in the sea. On average the pressure increases about 1.013 bar (1 atm) for every 10 m of depth. Related to the weight of overlying water at a given depth, hydrostatic pressure in the oceans ranges from 0 to more than 1013 bar (1000 atm). Thus, the highest pressures are experienced in the deep ocean trenches. Among the marine fauna, some members are adapted to live only in surface waters, others at intermediate depths, and still others at abyssal depths. Generally, none are known that can live over the entire depth range of the open ocean. Although microorganisms such as bacteria appear to be more adaptable to changes in hydrostatic pressure, facultative (pressure-tolerant) and obligately barophilic (pressure-requiring) bacteria are known (see also p. 92).

Salinity and temperature affect the *density* of seawater. At 0°C, seawater with a salinity of 30–37‰ has a corresponding density range of 1.024–1.030 g cm^{-3}. A variation in seawater density due to variation in salinity is one cause of water movement in the ocean, because denser water will sink below lighter water, or conversely, lighter water will rise above denser water (upwelling). The following processes may cause changes in salinity, and therefore, density: (1) dilution of seawater by runoff or less saline water; (2) dilution by rain or snow; (3) concentration through surface evaporation; (4) freezing, which excludes salts from ice and thus leaves any residual unfrozen water more saline; or (5) thawing of ice, which dilutes already existent saline water.

As already stated, variation in salinity of seawater is not the sole cause of variation in density. The other important cause of density variation of seawater is temperature. Unlike freshwater, whose density is greatest at 4°C (Fig. 5.6B),



(A)



(B)

FIG. 5.6 Density relationships in seawater and freshwater. (A) Relationship of seawater salinity to freezing point. (○) Temperature of maximum density at a given salinity; (●) freezing point temperature at a given salinity. Note that above a salinity of 24.7‰ seawater freezes at its maximum density because its temperature at maximum density cannot be lower than its freezing point. (B) Relationship of freshwater density (in $g\,cm^{-3}$) to temperature. Data points for chemically pure water are shown. Note that in the case of freshwater, its density at its freezing point is lower than its density at 4°C.

seawater with a salinity of 24.7‰ or greater has maximum density at its freezing point (Fig. 5.6A). A body of freshwater thus freezes from its surface downward because freshwater at its freezing point is lighter than at a temperature of 4°C. Ocean water in the Arctic or Antarctic seas also freezes from the surface downward, but in this instance because ice, which excludes salts as it forms from seawater, is lighter than the seawater and will thus float on it.

The temperature of seawater ranges from about −2° (the freezing point at 36‰ salinity) to +30°C, in contrast to the temperature of air over the ocean, which ranges from −65 to +65°C. The narrower temperature range for seawater can be related to (1) its heat capacity, (2) its latent heat of evaporation, and (3) heat transfer from lower to higher latitudes by surface currents in both hemispheres. The major source of heat in the ocean is solar radiation. More than half the surface waters of the ocean are at 15–30°C. Only 27% of the surface waters are below 10°C. From about 50° north latitude to 50° south latitude, the ocean is thermally stratified. In this range of latitudes, the seawater temperature below about 1000 m is below 4°C (deep water). At depths from about 300 to 1000 m, the temperature drops rapidly with increasing depth. The zone of this rapid temperature change is called the **thermocline**. Its thickness and position vary with geographic location and season of the year. Above the thermocline lies the warm surface water, the *mixed layer*, which is extensively agitated by wind and water currents and thus exhibits relatively little temperature change with increasing depth.

At latitudes higher than 50°N and 50°S, seawater is not thermally stratified. The waters around Antarctica, being cold (−1.9°C) and hypersaline (34.82‰) owing to ice formation, are hyperdense and thus sink below warmer, less dense water to the north and flow northward along the bottom of the ocean basin. This is an example of convergence. Similarly, Atlantic waters from the subarctic region having a temperature in the range of 2.8–3.3°C and a salinity in the range of 34.9–34.96‰ sink and flow southward at near bottom to bottom levels of the ocean. Because the Arctic Ocean bottom is separated from the other oceans by barriers such as the shallow Bering Strait in the case of the Pacific Ocean and a shallow ridge in the case of the Atlantic Ocean, it does not influence the water masses of the Pacific and Atlantic oceans directly. Other convergences occur in the world's oceans in both hemispheres because of interaction of waters of different densities. In these instances, the heavier waters sink to lesser depths because they have lower densities than the heavier waters at high latitudes.

The water convergences alluded to above help to explain why generally ocean water is oxygenated at all depths (Fig. 5.7). Of all ocean waters, only some coastal or near-coastal waters (e.g., estuarine waters; Cariaco Trench) may, as a result of intense biological activity, be devoid of oxygen at depth. At some sites, intense biological activity is sometimes the direct result of human pollution. Surface waters of the open ocean tend to be saturated with oxygen because of oxygenation by the atmosphere and, equally important, by the photosynthetic

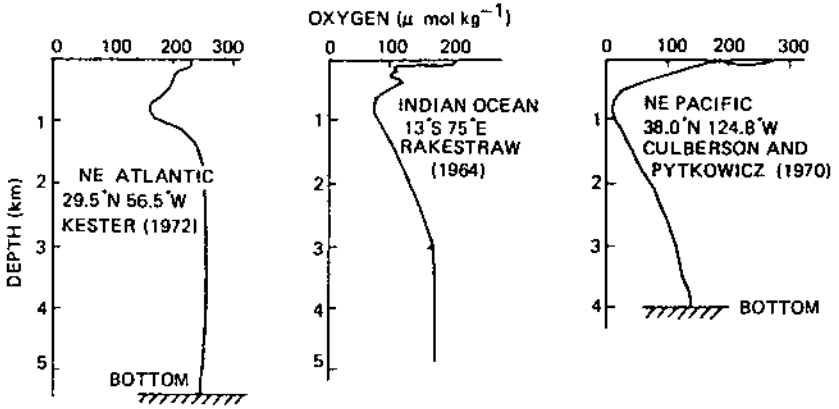


FIG. 5.7 Vertical distribution of oxygen in the ocean. Profiles from three ocean basins. [From Kester, 1975, with permission from Chemical Oceanography (JP Riley and G Shirrow, eds.) Vol. 1, 2nd ed., 1975. Copyright by Academic Press Inc., London Ltd.]

activity of the phytoplankton. Oxygenation by phytoplankton can occur to depths of about 100 m (200 m in exceptional cases), where light penetration is 1% of the surface illumination. Seawater at salinity of 34.352‰ is saturated at 5.86 mL or 8.40 mg of oxygen per liter at 760 mmHg and 15°C. The higher the salinity and the higher the temperature, the lower is the solubility of oxygen in seawater.

Starting at the top of a water column, the oxygen concentration in seawater will at first decrease with depth, owing mainly to oxygen consumption by the respiration of living organisms (Fig. 5.7). Because many life forms in the oceans tend to be concentrated in the upper waters, oxygen concentration will fall to a minimum at about 600–900 m of depth, where respiration (oxygen consumption) by zooplankton and other animal forms as well as bacterioplankton occurs but not photosynthesis (oxygen production) by phytoplankton. Below this depth, because of rapidly decreasing biological activity, the oxygen concentration may at first increase once more and then slowly decrease again toward the bottom. Bottom water, however, may still be half-saturated with oxygen relative to surface water. This oxygen is not supplied by in situ photosynthesis, which cannot occur in the absence of light at these depths, nor is it the result of significant oxygen diffusion from the atmosphere to these depths. As previously indicated, these oxygenated waters derive from the Antarctic and subarctic convergences. The oxygen-carrying waters from the Antarctic convergences flow northward along the bottom and at intermediate depths of the ocean basins whereas the the waters from the subarctic convergence in the Atlantic flow southward at more intermediate depths. The oxygen content of these waters is only slowly depleted because of the low numbers of oxygen-consuming organisms at these deep

regions of the oceans and the low rates of oxygen consumption in the upper sediments.

Photosynthetic activity of phytoplankton is dependent on penetration of sunlight into the water column because phytoplankton derives its energy almost exclusively from sunlight. It has been shown that light absorption by pure water in the visible range between 400 and 700 μm increases greatly toward the red end of the spectrum. It has also been shown that light that penetrates transparent water has been 60% absorbed at a depth of 1 m. The same light has been 80% absorbed at 10 m and 99% absorbed at 140 m. In less transparent coastal water, 95% of the light may have been absorbed at 10 m. Although the photosynthetic process of phytoplankters can use light over the entire visible spectrum, action spectra show peaks in the red and blue ends of the spectrum, where chlorophylls absorb optimally. Accessory pigments, such as carotenoids, absorb light at intermediate visible wavelengths. Clearly, light penetration limits the depth at which phytoplankton can grow. This depth is about 80–100 m on average (200 m maximally) and often much less in less transparent waters. Two exceptions have been noted, however. One was seen off the northern border of San Salvador Island in the Bahamas, where crustose coralline algae (Rhodophyta) were growing attached to rock at a depth of 268 m, observed from a submersible. At this location, the light intensity was only about 0.0005% of that at the surface (Littler et al., 1985). The other exception was noted in the Black Sea. Here the photosynthetic sulfur bacterium *Chlorobium phaeobacteroides* was found to grow in a chemocline at a depth of 80 m, where light transmission from surface irradiance has been calculated to be 0.0005% (Overmann et al., 1992), as at the station at San Salvador Island.

The water layer from the ocean surface to the depth below which photosynthesis cannot take place constitutes the **euphotic zone**. Zooplankton and bacteria, except for cyanobacteria, may abound to somewhat lower depths than phytoplankton (about 750 m), being scavengers and able to feed on dying and dead phytoplankters and their remains in the process of settling.

Microbial Distribution in Water Column and Sediments

Microbial distribution in the open oceans is not uniform throughout the water column (Fig. 5.8). Factors affecting this distribution are energy, carbon, nitrogen, and phosphorus limitations (e.g., Wu et al., 2000) and also temperature, hydrostatic pressure, and salinity. Accessory growth factors, such as vitamins, may also be limiting to those microbes that cannot synthesize them themselves. Phytoplankton distribution is limited to the euphotic zone of the water column primarily by available sunlight, the energy source for these organisms. However, phytoplankton distribution in the euphotic zone may also be limited by

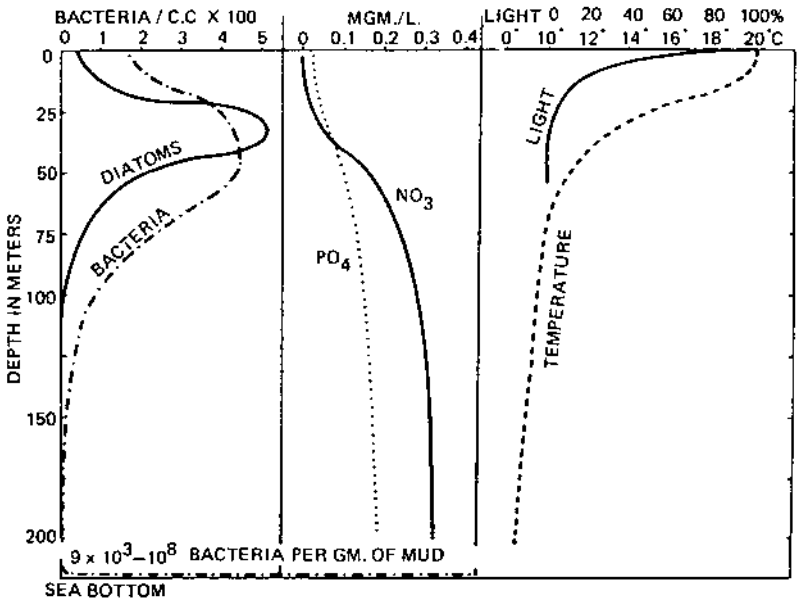


FIG. 5.8 Vertical distribution of bacteria (number per cubic centimeter of water), diatoms (number per liter of water), PO_4, NO_3 (milligrams per liter), light, and temperature in the sea based upon average results at several stations off the coast of southern California. (Reproduced with permission from CE ZoBell, Bacteria of the marine world, Scientific Monthly S5:320–330. Copyright 1942 by the American Association for the Advancement of Science.)

temperature and to some extent by salinity as well as by available dissolved nitrogen (nitrate) and phosphorus (phosphate).

Nonphotosynthetic microorganisms are limited to certain zones in the water column of the oceans by nutrient availability and in addition by temperature, salinity, and hydrostatic pressure. The nonphotosynthetic microorganisms include predators (zooplankton), scavengers (zooplankton, fungi), and decomposers (bacteria and fungi and, possibly to a small extent, zooplankton). Zooplankters therefore dominate the euphotic zone, where they can feed optimally on phytoplankton, zooplankton, and bacteria. Bacteria and fungi are also prevalent here, because they find sufficient sources of nutrients produced by the phytoplankton and zooplankton in secretions and excretions and in dead remains. Very high bacterial populations occur at the air/water interface of the ocean as a result of concentrations of organic carbon in the surface film that may exceed concentrations in the waters below by three orders of magnitude. The bacterial population in this film is known as **bacterioneuston** (Sieburth, 1976; Sieburth et al., 1976; Wangersky, 1976).

Marine sediments contain significant numbers of living bacteria, fungi, and other benthic microorganisms as well as higher forms of life. In 1940, Rittenberg reported the recovery of viable bacteria from 350 cm below the sediment surface in the Pacific Ocean (Rittenberg, 1940), a finding that at that time must have seemed remarkable and that ZoBell considered very significant (ZoBell, 1946; Ehrlich, 2000a). In 1994, Parks and colleagues reported finding bacteria at depths greater than 500 m below the sediment surface at five Pacific Ocean sites (Parks et al., 1994). The bacterial numbers decrease with depth in a sediment column. Fungi seem commonly to be restricted to sediments at shallow water depths, whereas bacteria, protozoa, and metazoa are found associated with sediments of shallow as well as abyssal depths. The chief function of the microbes is to aid in scavenging or decomposing organic matter that has settled undecomposed or partially decomposed from the overlying regions of biological productivity. Most of the organic matter from the euphotic zone settles to the bottom in the form of fecal pellets from metazoa. It should be pointed out that not all settled organic matter in deep sea sediments is utilizable by microbes, for reasons that are not yet clearly understood. This unutilizable organic matter constitutes a significant part of the *sedimentary humus*.

The metabolic activity of free-living bacteria of deep sea sediments has been shown to be at least 50 times lower than that of microorganisms in shallow waters or on sediments at shallow depths (Jannasch et al., 1971; Jannasch and Wirsen, 1973; Wirsen and Jannasch, 1974). Environmental factors contributing to this slow rate of bacterial metabolism seem to be the low temperature ($<5^{\circ}C$) and, especially, elevated hydrostatic pressure. Turner (1973) observed that pieces of wood left for 104 days on sediment at a station in the Atlantic Ocean at a depth of 1830 m was rapidly attacked by two species of wood borers (mollusks). This observation led to the suggestion that the primary attackers of organic matter in the deep sea, including sediment, are metazoa. Bacteria and other microbes harbored in the digestive tract of these metazoans decompose this organic matter only after ingestion by the metazoa (see, for instance, Jannasch, 1979). The intestinal bacteria appear essential to the digestion of cellulose to enable these metazoa to assimilate it.

Schwartz and Colwell (1976) were the first to report that the bacterial flora of the intestines in amphipods (crustaceans) that they collected in the Pacific Ocean at a depth of 7050 m was able to grow and metabolize nearly as rapidly at 780 atm and $3^{\circ}C$ as at 1 atm and $3^{\circ}C$ in laboratory experiments. Their study suggested that these types of gut bacteria behave very differently from free-living bacteria from the same depths. These findings have been extended by other observations on amphipod microflora. Deming and Colwell (1981) reported finding barophilic and barotolerant bacteria in the intestinal flora of amphipods living at 5200–5900 m. Yayanos et al. (1979) isolated a barophile from a

decomposing amphipod that grew optimally at ~500 bar and 2–4°C and poorly at atmospheric pressure in this temperature range. The same workers (Yayanos et al., 1981) isolated an obligately barophilic bacterium from an amphipod recovered from 10,746 m in the Marianas Trench.

The generally low rate of metabolism of the biological community (benthos) on deep sea sediments is also reflected by respiratory measurements carried out at 1850 m. The measurements revealed a rate of oxygen consumption that was orders of magnitude less than in sediments at shallow shelf depths (Smith and Teal, 1973).

Phytoplankton, zooplankton, bacteria, and fungi are not found in very significant numbers at intermediate depths of the oceans. The main reason for this is a lack of adequate nutrient supply, including a suitable source of energy, but low temperature can also be a factor in the case of some organisms. Kriss (1970), having examined water samples from a north-south transect in the Atlantic Ocean, concluded that an uneven distribution of bacteria at intermediate depths was attributable to the different origins and characteristics of particular water masses. He drew his conclusions on the basis of available metabolizable nutrients, claiming that higher concentrations occur in water masses of equatorial-tropical origin, owing to **autochthonous** (of native, e.g., planktonic, origin) and **allochthonous** (from runoff from continents and islands) contributions, than in water masses of Arctic and Antarctic origin.

Bacterial and fungal growth and reproduction in ocean water also occur on surfaces of some living organisms and on the surface of suspended organic and inorganic particles (epiphytes) because at these sites essential nutrients may be very concentrated (Sieburth, 1975, 1976; Hermansson and Marshall, 1985). The microorganisms may form microcolonies or biofilm on these surfaces. Detritus, even if by itself not a nutrient, usually has adsorptive capacity, which helps to concentrate nutrients on its surface and thus makes for a preferred microbial habitat. The beneficial effect that the buildup of nutrients by adsorption to particle surfaces has on microbial growth is great, because the concentration of these nutrients in solution in seawater is very low ($0.35–0.7\ mg\ L^{-1}$) (Menzel and Rhyter, 1970). ZoBell (1946) long ago showed a significant increase in the bacterial population in natural seawater during 24 h of storage in an Erlenmeyer flask. He attributed this to the adsorption of essential nutrients in the seawater to the walls of the flask, where the bacteria actually grew.

Effects of Temperature and Pressure on Microbial Distribution

Temperature and pressure may have a profound influence on where a given nonphotosynthetic microbe may live in the ocean. Some will grow only in the

temperature range of 15–45°C (**mesophiles**), others will grow only in the range from 0°C or slightly below to 20°C with an optimum at 15°C or below (**psychrophiles**), and still others will grow in the range of 0–30°C or even higher (e.g., 37°C) (Ehrlich, 1983) with an optimum near 25°C (**psychrotrophs**) (Morita, 1975). The mesophiles would be expected to grow only in waters of the mixed zone and near active hydrothermal vents, whereas psychrophiles would grow only below the thermocline and in polar seas. Psychrotrophs would be expected to grow above, in, and below the thermocline and in the polar seas, although they might do better in and above the thermocline. Mesophiles can be recovered from cold waters and deep sediments, where they are able to survive but cannot grow (i.e., they are **psychrotolerant**).

Many bacteria that normally grow at atmospheric pressure are not inhibited by hydrostatic pressures up to about 202.6 bar (200 atm) but are retarded at 303.9 bar (300 atm) and will not grow above 405.2 bar (400 atm). Many bacteria isolated from waters at 506.5 bar (500 atm) and 607.8 bar (600 atm) were found to grow better at these pressures under laboratory conditions than at atmospheric pressure. Such organisms are called **barophiles**. Some organisms described in a pioneering study by ZoBell and Morita (1957), which had been recovered from extreme depths (10,000 m), were suspected of having been obligate barophiles. Since that time, an obligately barophilic bacterium has actually been isolated from an amphipod taken at 10,476 m in the Marianas Trench and studied (Yayanos et al., 1981). It exhibited an optimal growth rate (generation time of 25 hr) at 2°C and 690 bar (681 atm) of hydrostatic pressure. As already mentioned, Yayanos et al. (1979) also isolated a facultative barophilic spirillum from 5700 m depth that grows fastest at about 500 bar (493.5 atm) and 2–4°C, with a generation time of 4–13 hr.

In prokaryotes, the most pressure-sensitive biochemical process is protein synthesis. It determines the degree of barotolerance and limits growth under pressure. Other processes, even nucleic acid synthesis, in the same cells are less pressure-sensitive (Pope and Berger, 1973). The most pressure-sensitive step in protein synthesis is translation, and the site of action is the 30S ribosomal subunit (Smith et al., 1975). (For a more complete discussion of ecological implications of temperature and pressure in the marine environment, see Marquis, 1982; Morita, 1967, 1980; and Jannasch and Wirsen, 1977.)

Marine microorganisms, especially bacteria, vary in their salinity requirements. Those that can grow only in a narrow range of salinities are said to be **stenohaline**, and those that can grow in a wide range of salinities are called **euryhaline**. Both types are found in the open ocean. Their salt requirement is not explained on the basis of osmotic pressure but by a specific requirement for one or more of the ions Na^+, K^+, Mg^{2+}, and Ca^{2+}. These ions may affect cell permeability or specific enzyme activities, or both (MacLeod, 1965). They may also affect cell integrity.

Dominant Phytoplankters and Zooplankters

Diatoms, dinoflagellates, coccolithophores, and other flagellates are the dominant phytoplankters of the sea (Fig. 5.9) (Sieburth, 1979). The first two are the chief source of food for herbivorous marine organisms. Diatoms are also important agents in the control of Si and Al concentrations in seawater (Mackenzie et al., 1978). Kelps and other sessile algae are mostly restricted to the shelf areas of the seas because they cannot grow at depths below about 30 m. A few kelps can float in the open ocean (e.g., Sargasso weed). The dominant members of zooplankton include not only protozoa but also invertebrates such as coelenterates, pteropods, and crustaceans, some of which are not found free-floating as adults but have planktonic larval stages. Among protozoa of the zooplankton, dominant forms include foraminifers and radiolarians. Their place in the ecology in the oceans is chiefly as predators on bacteria and some other members of the plankton population. Some of these forms also are the food for higher predatory animals. The phytoplankters are the principal **primary producers** (i.e., the chief synthesizers of organic carbon by photosynthesis) in the euphotic zone of the oceans. (For further discussion see Sieburth, 1979.)

At special sites at abyssal depths around hydrothermal vents or seeps from which H_2S or methane is discharged, primary production is the result of chemoautotrophic bacteria that obtain energy from the oxidation of hydrogen sulfide (Paull et al., 1984; Jannasch and Taylor, 1984; Stein, 1984, Tunnicliffe, 1992) or methane (Jannasch and Mottl, 1985; Kulm et al., 1986). This primary production is the basis for the existence of biological communities including metazoa and even vertebrates that are spatially restricted to the site of activity of the primary producers and their energy sources.

Plankters of Geomicrobial Interest

The phytoplankters of special geomicrobial interest include the diatoms, coccolithophores, and silicoflagellates. The zooplankters of special geomicrobial interest include the foraminifers and radiolarians. It is these organisms that precipitate much of the $CaCO_3$ and SiO_2 in the open sea. Their calcareous and siliceous remains, respectively, settle out and become incorporated into the sediments (see Chaps. 8 and 9).

Bacterial Flora

The bacterial flora of the seas are primarily represented by Bateria that are aerobic, facultative, and gram-negative (Dworkin, 1999) and Archaea, including Crenarchaeota and Euryarchaeota, which in general have not yet been cultured (Massana et al., 2000). Members of the genera *Shewanella, Pseudomonas, Alteromonas, Vibrio*, and *Oceanospirillum* are common, although many other

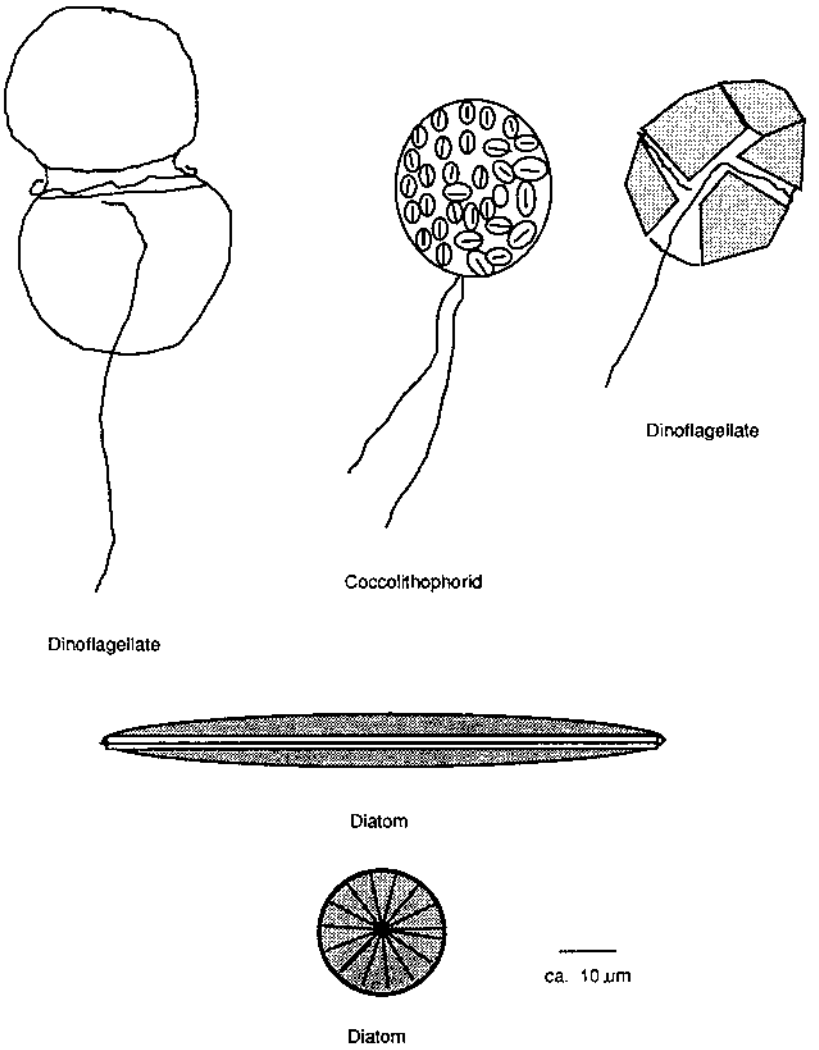


FIG. 5.9 Sketches of important marine phytoplankters. (Adapted from Wood, 1965, and Sieburth, 1979.)

gram-negative genera are also found, albeit in lesser numbers and in special niches. Gram-positive bacteria such as *Arthrobacter* or spore-forming rods are relatively less common and are encountered frequently in regions directly influenced by runoff from land. Most of the bacteria in the sea are aerobic or

facultative. Strict anaerobes, such as sulfate reducers, are encountered mainly where organic matter accumulates in significant quantities such as in salt marshes, estuaries, and some near-shore waters. Although heterotrophic and mixotrophic bacteria are the most numerous, autotrophic bacteria are encountered in significant numbers, particularly in certain niches. Chemosynthetic autotrophs are the primary producers around deep sea hydrothermal vents and may be free-living or occur as facultative or strict symbionts of some invertebrates. The latter reside, for instance, in **trophosomes** in the coelomic cavity of vestimentiferan worms and on the gills of certain mollusks. The cyanobacterium *Trichodesmium*, a photosynthetic autotroph, is widespead in the euphotic zone of the open ocean. Apart from being a photosynthetic primary producer, it is also an important nitrogen fixer in the sea (Zehr, 1992; Capone et al., 1997). Other cyanobacteria, such as *Oscillatoria*, *Lyngbia*, *Plectonema*, and *Spirulina*, are also encountered in the oceans. Nonsymbiotic heterotrophic, mixotrophic, or autotrophic bacteria in the marine environment may be free-living or attached to living organisms or to inert particles suspended in the seawater column (e.g., inorganic matter such as clay particles and fecal pellets) (for more information on bacterial attachment to fecal pellets, see Turner and Ferrante, 1979). They also can be detected in the sediment column in all parts of the ocean. Marine bacteria are usually defined as types that will not grow in media prepared in freshwater because they need one or more of the salt constituents of seawater. Bacteria that do not require seawater for growth can, however, be readily isolated from seawater and marine sediment samples far from any shore. Many of these organisms can grow readily in media prepared in seawater. They may represent terrestrial forms. The active bacteria in the marine environment are important as decomposers and in special marine niches as primary producers. Some may also play an important role in mineral formation and mineral diagenesis (e.g., ferromanganese deposits) (For further discussion see Ehrlich, 1975, 2000b; Jannasch, 1984; Kulm et al., 1986; Sieburth, 1979.)

5.2 FRESHWATER LAKES

Freshwater amounts to less than 3% of the total water on Earth. Like the oceans, it furnishes important habitats for certain life forms. Among these habitats are lakes, which are part of the **lentic** environments, the standing waters. Other lentic environments are ponds and swamps. Lakes represent only 0.009% of the total water in land areas (van der Leeden et al., 1990). Most of the freshwater is tied up in ice caps and glaciers (2.14%) and groundwater (0.61%) (van der Leeden et al., 1990).

Lakes have arisen in various ways. Some resulted from glacial action. An advancing glacier gouged out a basin that, when the glacier retreated, filled with

water from the melting ice and was later kept filled by runoff from the surrounding *watershed*. Other basins resulted from landslides that obstructed valleys and blocked the outflow from their watershed. Still others resulted from crustal up-and-down movement (dip-slip faulting) that formed dammed basins for the collection of runoff water. Some resulted from solution of underlying rock, especially limestone, which led to the formation of basins in which water collected. Lakes have also been formed by the collection of water from glacier melts in craters of extinct volcanoes and by the obstruction of river flow or changes in river channels (Welch, 1952; Strahler and Strahler, 1974; Skinner et al., 1999).

Lakes vary greatly in size. The combined Great Lakes in the United States cover an area of 328,000 km^2, an unusually great expanse. More commonly, lakes cover areas of 26–520 km^2, but many are smaller. Most lakes are less than 30 m deep. However, the deepest lake in the world, Lake Baikal in southern Siberia (Russia), has a depth of 1700 m. The average depth of the Great Lakes is 700 m, and that of Lake Tahoe on the California–Nevada border is 487 m. The elevation of lakes ranges from below sea level (e.g., the Dead Sea at the mouth of the Jordan River) to as high as 3600 m (Lake Titicaca in the Andes on the border between Bolivia and Peru).

Some Physical and Chemical Features of Lakes

Some of the water of lakes may be in motion, at least intermittently. Most prevalent are horizontal currents, which result from wind action and the deflecting action of shorelines. Vertical currents are rare in lakes of average or small size. They may result from thermal, morphological, or hydrostatic influences. Thermal influence can result in changes in water density such that heavier (denser) water sinks below lighter water. Morphological influence can result from rugged bottom topography, which may deflect horizontal water flow downward or upward. Hydrostatic influences can result from springs at the lake bottom that force water upward into the lake. Besides horizontal and vertical currents, return currents may occur as a result of water being forced against a shore by wind and piling up. Depending on the type of lake and the season of the year, only a portion of the total water mass of a lake, or all of it, may be circulated by the wind. (For a further discussion see Welch, 1952; Strahler and Strahler, 1974.)

The waters of lakes vary in composition from very low salt content (e.g., Lake Baikal) to a very high salt content (e.g., Dead Sea between Israel and Jordan and Lake Natron in Africa), and from low organic content to high organic content. Salt accumulation in lakes is the result of input of runoff from the watershed, including stream flow, very gradual solution of sediment components and rock minerals in the lake bed, and evaporation.

The waters of lakes may or may not be thermally stratified, depending upon various factors: geographic location, the season of the year, and lake depth and size. Thermal stratification, when it occurs, may or may not be permanent. When the waters are not stratified, wind action can cause complete mixing or *turnover*. When the waters are thermally stratified into a warmer layer in the upper portion of the lake (**epilimnion**) and a cooler layer in the lower portion (**hypolimnion**), complete mixing is not possible because of a density difference between the two layers. A thermocline is formed between the epilimnion and the hypolimnion, which is a relatively thin layer of water in which a temperature gradient appears with the temperature of the bottom of the epiliminion at the top and the temperature of the top of the hypoliminion at the bottom. Lakes may be classified according to whether and when they turn over (Reid, 1961). The categories can be defined as follows.

Amictic lakes are bodies of water that never turn over, being permanently covered by ice. Such lakes are found in Antarctica and at high altitudes in mountains.

Cold monomictic lakes are bodies of water that contain waters never exceeding 4°C, which turn over once during the summer, being thermally stratified the rest of the year.

Dimictic lakes turn over twice each year, in spring and fall. They are thermally stratified at other times. These are typically found in termperate climates and at higher altitudes in subtropical regions.

Warm monomictic lakes have water that is never colder than 4°C. They turn over once a year in winter and are thermally stratified the rest of the year.

Oligomictic lakes contain water that is significantly warmer than 4°C and turn over irregularly. Such lakes are found mostly in tropical zones.

Polymictic lakes have water just over 4°C and turn over continually. Such lakes occur at high altitude in equatorial regions.

Meromictic lakes are deep, narrow lakes whose bottom waters never mix with the waters above. The bottom waters usually have a relatively high concentration of dissolved salts, which makes them dense and separates them from the overlying waters by a chemocline. The upper waters in temperate climates may be thermally stratified in summer and winter and may undergo turnover in spring and fall.

A dimictic lake in a temperate zone during spring thaw accumulates water near 0°C, which, because of its lower density, floats on the remaining denser water, which is near 4°C. As the season progresses, the colder surface water is slowly warmed by the sun to near 4°C. At this point, all water has a more or less uniform temperature and thus uniform density. This allows the water to be completely mixed or turned over by wind agitation if the lake is not excessively deep like a meromictic lake. As the surface water undergoes further warming by

the sun, segregation of water masses recurs as warmer, lighter water comes to lie over colder, denser water. A thermocline is established between the two water masses, separating them into epilimnion and hypolimnion. The temperature of the water in the epilimnion may be greater than 10°C and vary little with depth (perhaps 1°C m^{-1}). The water in the thermocline, on the other hand, will show a rapid drop in temperature with depth. This drop may be as drastic as 18.3°C m^{-1} but is more usually 8°C m^{-1}. The thickness of the thermocline varies with position in the lake and between different lakes, an average value being around 1 m. The water in the hypolimnion will have a temperature well below that in the epilimnion and show a small drop in temperature with depth, usually less than 1°C m^{-1}.

The water in the epilimnion but not in the hypolimnion is subject to wind agitation and is thus fairly well mixed at all times. It is the greater density of the water of the hypolimnion that prevents its mixing by the wind. Continual warming by the sun and mixing by the wind produces horizontal currents and, in larger lakes, return currents over the thermocline, resulting in some exchange with water of the thermocline. This water exchange progressively increases the volume of the epilimnion and causes a progressive drop in the position of the thermocline.

At fall turnover, the thermocline will have touched bottom in the lake and disappeared, the water now having a uniform warm temperature. With the approach of winter, the lake water will cool. Once the surface water has cooled below 4°C, a thermocline will be re-established. Ice may form on the epilimnion if the water temperature reaches the freezing point. The winter thermocline will usually remain near the lower surface of any ice cover on the lake. Figure 5.10 shows in idealized form the seasonal cycle of thermal stratification of a dimictic lake.

The thermocline of a lake acts as a barrier between the epilimnion and the hypolimnion. It prevents easy exchange of salts, dissolved organic matter, and gases because the two water masses that it separates do not readily mix owing to their difference in density, and diffusion across the thermocline is very slow. The oxygen content in the epilimnion is usually around the saturation level. At times of intense photosynthetic activity of phytoplankton, oxygen supersaturation may be achieved. The source of oxygen in the epilimnion is photosynthesis and aeration, especially during wind agitation. The oxygen concentration in freshwater at saturation at 0°C is 14.62 ppm or 10.23 mL L^{-1}; at 15°C it is 10.5 ppm or 7.10 mL L^{-1}; and at 20°C it is 9.2 ppm or 6.5 mL L^{-1}. Optimal conditions of light and oxygen concentration together with adequate nutrient supply permit phytoplankton, zooplankton, bacteria, fungi, and other life to attain their greatest numbers in the waters of the epilimnion. The phytoplankters are the primary producers on which the remaining life forms depend directly or indirectly for food.

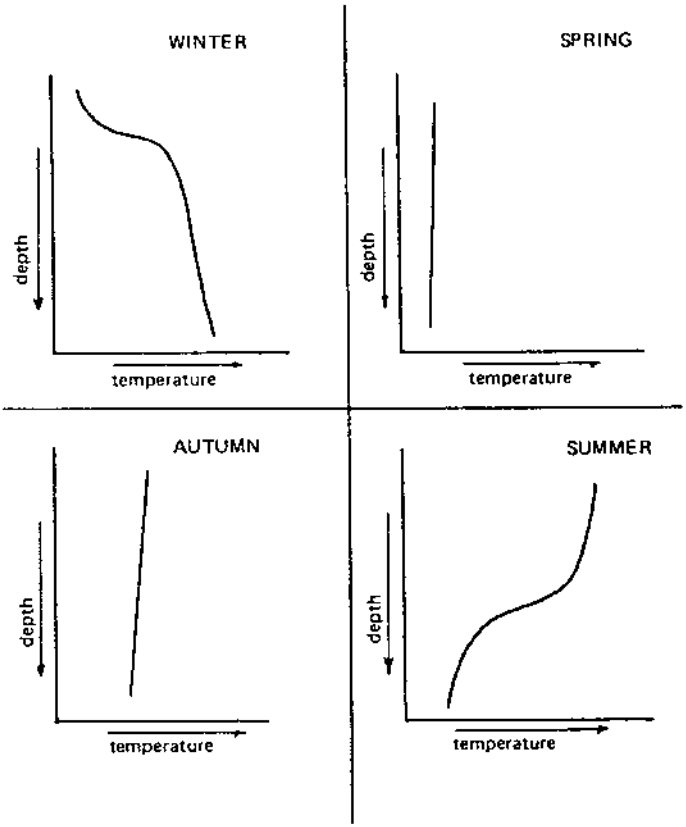


FIG. 5.10 Schematic representation of thermal stratification in a dimictic lake at different seasons of the year.

Oxygen that was distributed into all parts of the lake during spring turnover will be gradually depleted from the hypolimnion of a fertile (eutrophic) lake after re-emergence of thermal stratification. This is a consequence of biological activity, especially on and in the sediment. Thus, the hypolimnion may be anoxic during a shorter or longer period before fall turnover. Only anaerobic or facultative organisms will carry on active life processes under these anoxic conditions. Such organisms include bacteria and protozoa as well as certain nematodes, annelids, immature stages of certain insects, molluscs, and some fishes (Welch, 1952; Strahler and Strahler, 1974). Figure 5.11 illustrates the oxygen distribution measure in a dimictic lake during summer stratification.

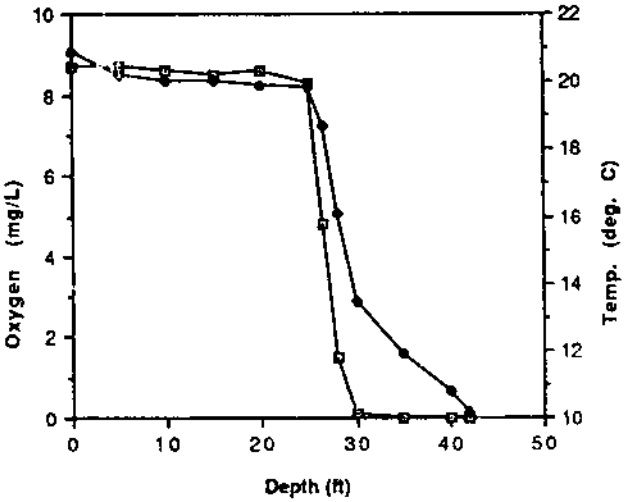


FIG. 5.11 Oxygen profile in Tomhannock Reservoir (near Troy, NY) on September 5, 1967. (□) Oxygen, mg L^{-1}. (◆) Temperature, °C. (From LaRock, 1969, with permission.)

Lake Bottoms

The nature of lake bottoms is highly variable, depending on the location and history of the lake. The basins of many smaller lakes are flat expanses of sediment overlying bedrock. On the other hand, the basins of larger lakes (e.g., the Great Lakes) have a more rugged topography in many places. The bottom of lakes may be dominated by sand and grit, by clay, by a brown, mud-rich humus, by diatom oozes, by ochreous mud rich in limonitic iron oxide, or by calcareous deposits. The organic components of any sediment may derive from dead or dying plankters that have settled to the bottom or from plant or animal remains. Some inorganic and some organic components may have been introduced into the lake and its sediment by the wind. Much silt and clay is washed into lakes by runoff. Some is also contributed by wind erosion of the shoreline. The sediments are a major habitat of microbes.

Lake Fertility

Lakes may be classified in terms of their fertility or their nutritional status (i.e., their ability to support a flora and fauna). **Oligotrophic** lakes have an impoverished nutrient supply in which phosphorus and/or nitrogen are in short supply and in which oxygen concentration is high at all depths. **Eutrophic** lakes, on the other hand, are fertile lakes in which phosphorus and nitrogen are in significantly

more plentiful supply than in oligotrophic lakes. **Mesotrophic** lakes are intermediate between oligotrophic and eutrophic lakes. **Dystrophic** lakes are defined as having an oversupply of organic matter, which cannot be completely decomposed because of an insufficiency of oxygen and alternative electron acceptors such as ferric iron, nitrate, or sulfate. They are sometimes deficient in assimilable nitrogen or phosphorus. The waters of such lakes are turbid and often acid. The origin of dystrophic conditions may be encroachment of the shoreline by plants, including reeds, shrubs, and trees.

Lake Evolution

Lakes have an evolutionary history. Once fully matured, they age progressively. Their basin slowly fills with sediment, partly contributed by the surrounding land through runoff and erosion and partly by the biological activity in the lake. The size of the contribution that each process makes depends on the fertility of the lake. Changes in climate may also contribute to lake evolution (e.g., through lessened rainfall, which can cause a drop in water level, or through increase in temperature, which can cause more rapid water evaporation). These effects usually make themselves felt slowly. Ultimately a lake may change into a swamp.

Microbial Populations in Lakes

The microbial population in eutrophic lakes tends to be orders of magnitude greater than in the seas. Numbers of culturable bacteria may range from 10^2 to 10^5 per milliliter of lakewater and be of the order of 10^6 per gram of lake sediment. The size of the bacterial population may be affected by runoff, which contributes soil bacteria. The culturable bacterial population of lakes consists predominantly of gram-negative rods (Wood, 1965, p. 36), although gram-positive spore-forming bacteria and actinomycetes can be readily isolated, especially from sediments. Few, if any, of the types of bacteria found in lakes are exclusively limnetic organisms. The main activity of the bacteria, other than the cyanobacteria, is that of decomposers. The cyanobacteria along with the algae serve as the primary producers. Fungi and protozoa are also found. Important functions of the former are as scavengers and decomposers and of the latter as predators.

Cyanobacteria and algae are abundant in eutrophic lakes. The algae include green forms as well as diatoms and pyrrhophytes. *Cyanobacterial* and *algal blooms* may occur at certain times when one species suddenly multiplies explosively and becomes the dominant phytoplanktonic member temporarily, often forming a carpetlike layer or mat on the water surface. After having reached a population peak, most of the cells in the phytoplankton bloom die off and are

attacked by scavengers and decomposers. Especially favorable growth conditions appear to be the stimulus for such blooms.

5.3 RIVERS

Rivers, which account for only 0.0001% of the total water in the world (van der Leeden et al., 1990), are part of the **lotic** environment in which waters move in channels on the land surface. Such flowing water may start as a brook, then widen into a stream and ultimately into a river. The source of the water is surface runoff and groundwater reaching the surface through springs or, more important, through general seepage. A riverbed is shaped and reshaped by the flowing water that scours the bottom and sides with the help of suspended particles from clays to small stones. Young rivers may feature rapids and steep valley slopes. Mature rivers lack rapids and feature more uniform stream flow, owing to a smoothly graded river bottom and an ever-widening riverbed. Old rivers may develop meanders in the wide, flat floodplains. The flow of the water is caused by gravity because the head of a river always lies above its mouth. Average flow rates of rivers range from 0 to 9 m sec^{-1}. However, the flow of water in a river cross section is not uniform. Some portions in such a section flow much faster than others. This can be attributed to frictional effects related to the riverbed topography as well as to density differences of different parts of the water mass. Density variations may arise from temperature differences or from solute concentration differences between parts of a river. Portions of river water may exhibit strong turbulence caused in part by certain features of the river topography. Water velocity, turbulence, and terrain determine the size of particles a river may sweep along (see Strahler and Strahler, 1974; Stanley, 1985; Skinner et al., 1999).

Most river water is ultimately discharged into an ocean, but exceptions exist. The Jordan River, whose headwaters originate in the mountains of Syria and Lebanon, empties into a lake called the Dead Sea, which has no connection with any ocean. The Dead Sea does not overflow because it loses its water by evaporation, which accounts, in part, for its high salt accumulation. Its waters are nearly saturated with salts, which makes life impossible except for specially adapted organisms. When river water is discharged into an ocean, an estuary is frequently formed where the less dense river water will flow over the denser saline water from the sea with incomplete mixing. Tidal effects of the sea may alter the water level of the discharging river, sometimes to a considerable distance upstream. Estuaries form special habitats for microbes and higher forms of life, which must cope with periodic changes in salinity, water temperature, nutrient and oxygen availability, and so forth, engendered by tidal movement.

Because of constant water movement, the water temperature of rivers tends to be rather uniform (i.e., rivers generally are not thermally stratified when examined in cross section). Only where a tributary at a different water temperature enters a stream may there be local temperature stratification. Different segments of a river may, however, differ in temperature. The pH of river water can range from very acid (pH 3), for instance in streams receiving acid mine drainage, which is the result of microbial activity, to alkaline (pH 8.6) (Welch, 1952, p. 413). Unless heavily polluted by human activities, rivers are generally well aerated. It has been thought that in unpolluted rivers most organic and inorganic nutrients supporting microbial as well as higher forms of life are largely introduced by runoff (allochthonous). It is now believed that a significant portion of fixed carbon in such streams and rivers may be contributed by autotrophs, mainly algae, growing in quiet waters (autochthonous) (Minshall, 1978). Pollution may cause organic overloading, which, because of excessive oxygen demand, will result in anoxic conditions with the consequent elimination of many micro- as well as macroorganisms.

Planktonic organisms tend to be found in greater numbers in the more stagnant or slower flowing waters of a river than in fast-flowing portions. The plankters include algae such as diatoms, cyanobacteria, green algae, protozoa, and rotifers. The proportions depend on the conditions of a particular river and its sections. Sessile plants or algae tend to develop to significant extents only in sluggish streams or in the backwaters of otherwise rapidly flowing streams. Bacteria are represented in significant numbers where physical and chemical conditions favor them. Rheinheimer (1980) reported total bacterial numbers in the River Elbe in Germany to range from 4.7×10^9 to 6.9×10^9 L^{-1} and bacterial biomass to range from 0.55 to 0.71 mg L^{-1} in an unspecified year. As in lakes, no unique microflora occurs in unpolluted rivers.

5.4 GROUNDWATERS

Water that collects below the land surface in soil, sediment, and permeable rock stata is called *groundwater*. It represents 0.61% of all water in the world (van der Leeden et al., 1990). Groundwater derives mainly from *surface water* whose origin is meteoric precipitation such as rain and melted snow. Surface water includes the water of rivers, lakes, and the like (Fig. 5.12). A minor amount of groundwater derives from **connate water**, **water of dehydration**, or **juvenile water**. Connate water, often of marine origin and therefore saline, is water that became trapped in rock strata in the geologic past by up or down warping or faulting and as a consequence became isolated as a stagnant reservoir. Its salt composition frequently became highly altered from that of the original water from which it derived as a result of long-term interaction with the enclosing rock.

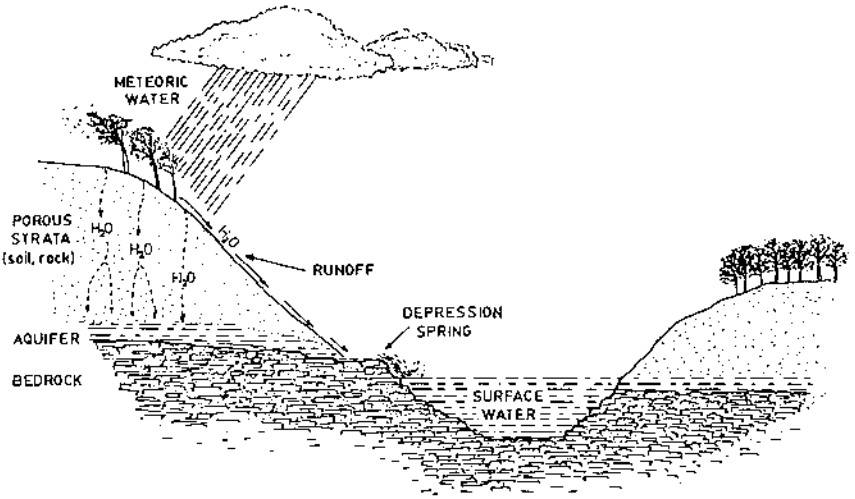


FIG. 5.12 Interrelationship of meteoric, surface, and ground waters.

Connate waters are often associated with oil formations. Waters of dehydration are derived from waters of crystallization, which are part of the structure of certain crystalline minerals. They are released as a result of the action of heat and pressure in the lithosphere. Juvenile waters are associated with magmatism, which causes them to escape from the interior of the Earth. They had never before reached the Earth's surface.

Surface water slowly infiltrates permeable ground as long as the ground is not already saturated. It passes through a zone of aeration or unsaturation called the *vadose zone* to the zone of saturation or *aquifer*, which lies over an impermeable rock stratum (Strahler and Strahler, 1974; Chapelle, 1993; Skinner et al., 1999). The vadose zone may include the soil, an intermediate zone, and the capillary fringe and can range in thickness from a few centimeters to 100 m or more. The resident water in the vadose zone is pore water (pellicular water; see Chap. 4) that is under less than atmospheric pressure and held there by capillarity. In soil this water supports the soil micro- and macroflora and fauna and plant growth. The water in the aquifer can be extracted through human intervention, but that in the vadose zone cannot (Hackett, 1972). The rate of infiltration of permeable strata depends not only on the surface water supply but also on the porosity of the permeable strata. Similarly, the water-holding capacity of the aquifer depends on the porosity of its matrix rock. The cause of water movement below ground level is not only gravity but also intermolecular attraction between water molecules, capillary action, and hydrostatic head (Chap. 4).

At a given location, two or more aquifers may occur, one over the other separated by one or more impervious strata. An example of such multiple aquifers is to be found in the Upper Atlantic Coastal Plain Province in South Carolina (Sargent and Fliermans, 1989). In such a sequence of aquifers, the uppermost may be directly rechargeable with surface water from above and is then called an *unconfined aquifer*, or it may be separated from a larger aquifer just below by a thin lens of material with low or no porosity, called an *aquiclude*, in which case it is called a *perched aquifer*. In the latter case, the lens material may be clay or shale. The underlying aquifers are rechargeable only where the overlying confining stratum is absent. They are called *confined aquifers*. The matrix of aquifers may consist of sand and gravel, limestone and other soluble rock, basalt and other volcanic rocks, sandstone and conglomerate, crystalline and metamorphic rocks, or other porous but poorly permeable materials (Hackett, 1972).

Groundwater may escape to the surface or into the atmosphere through *springs* or by evaporation with or without the mediation of plants (transpiration). Some water will be accumulated by the vegetation itself. Depending on the relative rates of water infiltration and water loss, the level of the *water table* in the ground may rise, fall, or remain constant. Groundwater that reaches the surface through springs may do so under the influence of gravity, which may create sufficient head to force the water to the surface through a channel, as in an *artesian spring*. Groundwater may also reach the surface as a result of an intersection of the water table with the land surface, as in a *depression spring*. Finally, groundwater may reach the surface in springs under the influence of thermal energy applied to reservoirs deep underground. Such *hot springs* in their most spectacular form are *geysers* from which hot water spurts forth intermittently. Some hot springs emit not only water derived from infiltration of surface water but also juvenile water.

As it infiltrates permeable soil and rock strata, surface water will undergo changes in the composition of dissolved and suspended organic and inorganic matter. These changes are the result of adsorption and ion exchange by surfaces of soil and rock particles. They are also the result of biochemical action of microbes, including bacteria, fungi, and protozoa, which exist mainly in biofilms on the surface of many of the rock particles and metabolize the adsorbed organic and (to a limited extent) inorganic matter (Cullimore, 1992; Costerton et al., 1994). Plant roots affect the solute composition in the rhizosphere through absorption and/or excretion. Polluted water infiltrating the ground may become thoroughly purified, provided that it moves through a sufficient depth and does not encounter major cracks and fissures, which, because of reduced surface area, would exert only limited "filtering" action. Under some circumstances, the groundwater may also become highly mineralized during filtration or after reaching the water table. If such mineralized water reaches the land surface, it

may leave extensive deposits of calcium carbonate, iron oxide, or other material as it evaporates.

Systematic studies of groundwater microbes are now being undertaken. As many as 10^6 bacteria per gram have been recovered from the vadose zone and some shallow water table aquifers. They included gram-positive and gram-negative types, the former in apparently greater numbers (Wilson et al., 1983). Evidence of fungal spores and yeast cells was also seen in one instance (Ghiorse and Balkwill, 1983), but eukaryotic microbes were generally thought to be absent from subsurface samples associated with groundwater. Sinclair and Ghiorse (1987) showed that the number of protozoa, mostly flagellates and amoebae, decreased sharply to 28 g^{-1} (dry weight) with increasing depth in the vadose zone at the bottom of a clay loam subsoil material taken from a site in Lula, Oklahoma. They were absent from the saturated zone except in gravelly, loamy sand matrix at a depth of 7.5 m, which also contained significant numbers of bacteria.

Special drilling methods that minimize the possibility of microbial contamination have been developed (see Fredrickson et al., 1993; Russell et al., 1992) for studying the microbiology of vadose zones and aquifers in the deep subsurface. Fredrickson et al. (1991) reported as many as 10^4–10^6 colony-forming heterotrophic bacteria per gram of Middendorf (~366–416 m deep) and Cape Fear sediments (~457–470 m deep) of Cretaceous age, which were obtained by drilling into the Atlantic Coastal Plain at Savannah River Plant, South Carolina. The isolates from the two sediments were physiologically distinct. Contrary to what they expected from their study of a shallow aquifer at Lula, Oklahoma (see above), Sinclair and Ghiorse (1989) found significant numbers of fungi and protozoa in samples from deep aquifer material from the Upper Atlantic Coastal Plain Province. The numbers of these organisms were highest where the prokaryotic population was high. The bacteria in the samples included members of the Bacteria and Archaea. Diverse physiological groups were represented, including autotrophs such as sulfur oxidizers, nitrifiers, and methanogens (Fredrickson et al., 1989; Jones et al., 1989) as well as heterotrophs such as aerobic and anaerobic mineralizers. The latter included denitrifiers, sulfate reducers, and Fe(III) reducers (Balkwill, 1989; Hicks and Fredrickson, 1989; Phelps et al., 1989; Madsen and Bollag, 1989; Francis et al. 1989; Chapelle and Lovley, 1992). Hydrogen- and acetate-oxidizing methanogens were also found (Jones et al., 1989). Rates of metabolism in deep aquifers of the Atlantic Coastal Plain have been estimated to range from 10^{-4} to 10^{-6} mmol of CO_2 per liter per year, using a method of geochemical modeling of groundwater chemistry (see also Chap. 4) (Chapelle and Lovley, 1990).

Significant numbers of autotrophic methanogens and homoacetogens have been detected in groundwater from the deep granitic aquifers (112–446 m) at the Äspö Hard Rock Laboratory in Sweden (Kotelnikova and Pedersen, 1998).

According to the investigators, these organisms may be the primary producers in this aquifer, using available hydrogen as their energy source. On the other hand, only $0–4.5 \times 10^{-1}$ autotrophic methanogens and $0–2.2 \times 10^{1}$ homoacetogens per milliliter were found in groundwater samples from deep igneous rock aquifers in Finland (Haveman et al., 1999). These samples did contain 0 to $> 1.6 \times 10^{4}$ sulfate reducers and 7.0×10^{0} to 1.6×10^{4} iron(III) reducers. A subsurface paleosol sample from a depth of 188 m in the Yakima Barricade Borehole at the U.S. Department of Energy's Hanford Site in the state of Washington yielded a variety of bacteria, including members of the Bacteria and Archaea. The members of the Bacteria included relatives of *Pseudomonas*, *Bacillus*, *Micrococcus*, *Clavibacter*, *Nocardioides*, *Burkholderia*, *Comamonas*, and *Erythromicrobium* in addition to six novel types with some affinity to the Chloroflexaceae. The members of the Archaea showed an affinity for the Crenarchaea branch (Chandler et al., 1998). The paleosol in which these bacteria were detected is part of a sedimentary formation overlying the Columbia River Basalt. It has been proposed that methanogens and homoacetogens along with sulfate reducers and iron(III) reducers, present in groundwater from aquifers in the Columbia River Basalt, form a biocenosis that uses H_2 generated in an interaction between water and ferromagnesian silicates in basalt as its energy source (Stevens and McKinley, 1995; Stevens, 1997). However, the occurrence of a reaction between ferromagnesian silicates in basalt and water has been questioned (Madsen, 1996; Lovley and Chapelle, 1996; Stevens and McKinley, 1996; Anderson et al., 1998). Indeed, although it can be shown on a thermodynamic basis that H_2 can be formed in a reaction between Fe^{2+} and water at pH 7.0,

$$3Fe^{2+} + 4H_2O \rightarrow H_2 + Fe_3O_4 + 6H^+ \quad (\Delta G_r^{o'} = -12.04 \text{ kcal or } -50.33 \text{ kJ}) \quad (5.3)$$

it cannot be formed in a reaction between ferrous silicate and water at pH 7.0, e.g.,

$$3FeSiO_3 + H_2O \rightarrow H_2 + Fe_3O_4 + 3SiO_2 \quad (\Delta G_r^{o'} = +12.59 \text{ kcal or } +52.63 \text{ kJ}) \quad (5.4)$$

The hydrogen in the Columbia River Basalt may come from other sources, as pointed out by Madsen (1996) and Lovley and Chapelle (1996).

Much remains to be learned about the microbial populations of pristine aquifers and their associated vadose zones and the response of this population to environmental stresses (pollution).

5.5 SUMMARY

The hydrosphere is mainly marine. It occupies more than 70% of the Earth's surface. The world's oceans reside in basins surrounded by the margins of continental land masses that project into the sea by way of the continental shelf, the continental slope, and the continental rise, bottoming out at the ocean floor. The ocean floor is traversed by mountain ranges cut by fracture zones and rift valleys—the mid-ocean spreading centers—where new ocean floor is being formed. The ocean floor is also cut by deep trenches, the zones of subduction, where the margin of an oceanic crustal plate slips beneath a continental crustal plate. Parts of the ocean floor also feature isolated mountains that are live or extinct volcanoes and may project above sea level as islands. The average world ocean depth is 3975 m; the greatest depth is about 11,000 m in the Marianas Trench.

Most of the ocean floor is covered by sediment of 300 m average thickness, accumulating at rates of less than 1 cm to greater than 10 cm per 1000 years. Ocean sediments may consist of sand, silt, and/or clays of terrigenous origin and of oozes of biogenic origin, such as diatomaceous, radiolarian, or calcareous oozes.

Different parts of an ocean are in motion at all times, driven by wind stress, the Earth's rotation, density variations, and gravitational effects exerted by the sun and moon. Surface, subsurface, and bottom currents have been found in various geographical locations.

Where water masses diverge, upwelling of deep waters occurs, which replenishes nutrients for plankton in the surface waters. Where the water masses converge, surface water sinks and carries oxygen to deeper levels of the ocean, ensuring some degree of oxygenation at all levels.

Seawater is saline (average salinity about 35‰) owing to the presence of chloride, sodium, sulfate, magnesium, calcium, potassium, and some other ions. Variations in total salt concentrations affect the density of seawater, as does variation in water temperature. The ocean is thermally stratified between 50° north and 50° south latitude into a mixed layer (about 300 m deep), with water at more or less uniform temperature between 15° and 30°C depending on latitude; a thermocline (about 700 m deep) in which the temperature drops to about 4°C with depth; and the deep water (from the thermocline to the bottom), where the temperature is uniform between less than 0° and 4°C. Hydrostatic pressure in the water column increases by about 1.013 bar (1 atm) for every 10 m of increase in depth. Light penetrates to an average depth of about 100 m, which restricts phytoplankton to shallow depths. Zooplankton and bacterioplankton can exist at all depths but are found in greatest numbers at the seawater/air interface, near where the phytoplankton abounds, and on the ocean sediment. Intermediate depths are at most sparsely inhabited because of limited nutrient supply.

Marine phytoplankton is constituted of algal forms, mainly diatoms, dinoflagellates, and coccolithophores, whereas marine zooplankton is constituted mainly of flagellated and ameboid protozoa as well as some small invertebrates. Bacterioplankton is composed of bacteria, chiefly heterotrophs, and of archaea, about whose traits we know little. Phytoplankton organisms are the primary producers, the zooplankton organisms are the predators and scavengers, and the heterotrophic bacteria are the decomposers. The metabolic rate of microorganisms decreases markedly with depth, probably as a result of the effects of high hydrostatic pressure and low temperature. Different life forms in the ocean show different tolerances to salinity, temperature, and hydrostatic pressure.

Freshwater is found in lakes and streams above ground and in saturated and unsaturated strata below ground. Lakes are standing bodies of water, usually of low salinity, which may be thermally stratified into epilimnion, thermocline, and hypolimnion. The degrees of stratification may vary with the season of the year. Water below the thermocline (i.e., the hypolimnion) may develop anoxia because the thermocline is an effective barrier to diffusion of oxygen into it. Only after the disappearance of the thermocline do these waters become reoxygenated due to total mixing by wind agitation. Lakes vary in their nutrient quality. Phosphorus is usually the most limiting element to lake life. Phytoplankton, zooplankton, and bacterioplankton are important life forms in lakes. Phytoplankton is restricted to the epilimnion, whereas zooplankton and bacterioplankton together with fungi are found in the entire water column and in the sediment.

Rivers constitute moving freshwater. They are generally not thermally stratified. Abundant life forms, such as phytoplankton, zooplankton, and bacterioplankton are concentrated mainly in the quieter portions of the streams, especially those that are not polluted.

Groundwaters are derived from surface waters that seep into the ground and accumulate as aquifers above impervious rock strata. Water from an aquifer may come to the surface again by way of springs or seepage. In passing through the ground, water is purified. Microorganisms as well as organic and inorganic chemicals are removed by adsorption to rock and soil particles. Organic matter may be mineralized by the microbial decomposers. Sediment samples from shallow as well as deep aquifers have revealed the presence of a significant microbial population with very diverse physiological potentials.

REFERENCES

Anderson RT, Chapelle FN, Lovley DR. 1998. Evidence against hydrogen-based microbial ecosystems in basalt aquifers. Science 281:976–977.

Balkwill DL 1989. Numbers, diversity, and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. Geomicrobiol J 7:32-52.

Bischoff JL, Rosenbauer RJ. 1983. A note on the chemistry of seawater in the range of 350°–500°C. Geochim Cosmochim Acta 47:139–144.
Bowden KF. 1975. Oceanic and estuarine mixing processes. In: Riley JP, Skirrow G, eds. Chemical Oceanography, Vol. 1. 2nd ed. London: Academic Press, pp 43–72.
Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ. 1997. *Trichodesmium*, a globally significant marine cyanobacterium. Science 276:1221–1230.
Chandler DP, Brockman FJ, Bailey TJ, Fredrickson JK. 1998. Phylogenetic diversity of archaea and bacteria in a deep subsurface paleosol. Microb Ecol 36:37–50.
Chapelle FH. 1993. Groundwater Microbiology and Geochemistry. New York: Wiley.
Chapelle FH, Lovley DR. 1990. Rates of microbial metabolism in deep coastal plain aquifers. Appl Environ Microbiol 56:1865–1874.
Chapelle FH, Lovley DR. 1992. Competitive exclusion of sulfate reduction by Fe(III) reducing bacteria: A mechanism for producing discrete zones of high-iron groundwater. Ground Water 30:29–36.
Church MJ, Hutchins DA, Ducklow HW. 2000. Limitation of bacterial growth by dissolved organic matter and iron in the southern ocean. Appl Environ Microbiol 66:455–466.
Coale KH, Johnson KS, Fitzwater SE, Gordon RM, Tanner S, Chavez FP, Ferioli L, Sakamoto C, Rogers P, Millero F, Steinberg P, Nightingale P, Cooper D, Cochlan WP, Landry MR, Constantinou J, Rollwagen G, Trasvina A, Kudela R. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature (Lond) 383:495–501.
Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G. 1994. Biofilms, the customized microniche. J Bacteriol 176:2137–2142.
Cullimore DR. 1992. Practical Groundwater Microbiology. Chelsea, MI: Lewis.
Defant A. 1961. Physical Oceanography, Vol. 1, Oxford, UK: Pergamon Press.
Deming JW, Colwell RR. 1981. Barophilic bacteria associated with deep-sea animals. BioScience 31:507–511.
Dietrich G, Kalle K. 1965. Allgemeine Meereskunde. Eine Einführung in die Ozeanographie. Berlin: Gebrüder Bornträger.
Dworkin, M. 1999. The Prokaryotes. An Evolving Electronic Resource for the Microbiological Community. Online Edition, New York: Springer-Verlag.
Edmond JM, Von Damm KL, McDuff RE, Measures CI. 1982. Chemistry of hot springs on the East Pacific Rise and their effluent dispersal. Nature (Lond) 297:187–191.
Ehrlich HL. 1975. The formation of ores in the sedimentary environments of the deep sea with microbial participation: The case for ferromanganese concretions. Soil Sci 119:36–41.
Ehrlich HL. 1983. Manganese oxidizing bacteria from a hydrothermally active area on the Galapagos Rift. Ecol Bull (Stockholm) 35:357–366.
Ehrlich HL. 2000a. ZoBell and his contributions to the geosciences. In: Bell CR, Brylinsky M, Johnson-Green, eds. Microbial Biosystems: New Frontiers. Proc 8th Int Symp Microb Ecol. Halifax, NS: Atlantic Canada Society for Microbial Ecology, pp 57–62.
Ehrlich HL. 2000b. Ocean manganese nodules: Biogenesis and bioleaching possibilities. Minerals Metallurgical Process 17:121–128.
Francis AJ, Slater JM, Dodge CJ. 1989. Denitrification in deep subsurface sediments. Geomicrobiol J 7:103–116.

Fredrickson JK, Garland TR, Hicks RJ, Thomas J:M, Li SW, McFadden KM. 1989. Lithotrophic and heterotrophic bacteria in deep subsurface sediments and the relationship to sediment properties. Geomicrobiol J 7:53–66.

Fredrickson JK, Balkwill DL, Zachara JM, Li S-MW, Brockman FJ, Simmons MA. 1991. Physiological diversity and distributions of heterotrophic bacteria in deep Cretaceous sediments in the Atlantic Coast Plain. Appl Environ Microbiol 57:402–411.

Fredrickson JK, Brockman FJ, Bjornstad BN, Long PE, Li SW, McKinley JP, Wright JV, Conca JL, Kieft TL, Balkwill DL. 1993. Microbial characteristics of pristine and contaminated deep vadose sediments from an arid region. Geomicrobiol J 11:95–107.

Gelder, RJ. 1999. Complex lessons of iron uptake. Nature (Lond) 400:815–816.

Ghiorse WC, Balkwill DL. 1983. Enumeration and morphological characterization of bacteria indigenous to subsurface environments. Dev Ind Microbiol 24:213–224.

Gross MG. 1982. Oceanography: A View of the Earth. 3rd ed. Englewood Cliffs, NJ: Prentice-Hall.

Guan LL, Kanoh K, Kamino K. 2001. Effect of exogenoue siderophores on iron uptake activity of marine bacteria under iron-limited conditions. Appl Environ Microbiol 67:1710–1717.

Hackett OM. 1972. Groundwater. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser Vol IVA. New York: Van Nostrand Reinhold, pp 470–478.

Haveman SA, Pedersen K, Ruotsalainen P. 1999. Distribution and metabolic diversity of microorganisms in deep igneous rock aquifers of Finland. Geomicrobiol J 16:277–294.

Hermansson M, Marshall KC. 1985. Utilization of surface localized substrate by non-adhesive marine bacteria. Microb Ecol 11:95–105.

Hicks RJ, Fredrickson JK. 1989. Aerobic metabolic potentials of microbial populations indigenous to deep subsurface environments. Geomicrobiol J 7:67–77.

Horn DR, Horn BM, Delach MN. 1972. Sedimentary Provinces, North Pacific (Map). Palisades, NY: Lamont-Doherty Observatory of Columbia University.

Hutchins DA, Witter AE, Butler A, Luther GW III. 1999. Competition among marine phytoplankton for different chelated iron species. Nature (Lond) 400:858–861.

Jannasch HW. 1979. Microbial turnover of organic matter in the deep sea. BioScience 29:228–232.

Jannasch HW. 1984. Microbial processes at deep sea hydrothermal vents. In: Rona PA, Bostrom K, Laubier L, Smith KL Jr., eds. Hydrothermal Processes at Seafloor Spreading Centers. New York: Plenum Press, pp 677–709.

Jannasch HW, Mottl MJ. 1985. Geomicrobiology of the deep-sea hydrothermal vents. Science 229:717–725.

Jannasch HW, Taylor CD. 1984. Deep-sea microbiology. Annu Rev Microbiol 38:487–514.

Jannasch HW, Wirsen CO. 1973. Deep-sea microorganisms: In situ reponse to nutrient enrichment. Science 180:641–643.

Jannasch HW, Wirsen CO. 1977. Microbial life in the deep sea. Sci Am 236:2–12.

Jannasch HW, Eimhjellen K, Wirsen CO, Farmanfarmaian A. 1971. Matter in the deep sea. Science 171:672–675.

Jones RE, Beeman RE, Suflita JM. 1989. Anaerobic metabolic processes in deep terrestrial subsurface. Geomicrobiol J 7:117–130.

Kester DR. 1975. Dissolved gases other than CO_2. In: Riley JP, Skirrow G, eds. Chemical Oceanography, Vol. 1. 2nd ed. New York: Academic Press, pp 497–547.
Kotelnikova S, Pedersen K. 1998. Distribution and activity of methanogens and homoacetogens in deep granitic aquifers at Äspö Hard Rock Laboratory, Sweden. FEMS Microbiol Ecol 26:121–134.
Kriss, AE. 1970. Ecological-geographic patterns in the distribution of heterotrophic bacteria in the Atlantic Ocean. Mikrobiologiya 39:362–371 (Engl transl pp 313–320).
Kulm LD, Suess E, Moore JC, Carson B, Lewis BT, Ritger SD, Kado DC, Thornburg TM, Embley RW, Rugh WD, Massoth GJ, Langseth MG, Cochrane GR, Scamman RL. 1986. Oregon subduction zone: Venting, fauna, and carbonates. Science 231:561–566.
LaRock PA. 1969. The bacterial oxidation of manganese in a fresh water lake. PhD Thesis. Rensselaer Polytechnic Institute, Troy, NY.
Littler MM, Littler DS, Blair SM, Norris JN. 1985. Deepest known plant life discovered on an uncharted seamount. Science 227:57–59.
Lovley DR, Chapelle FH. 1996. Technical comment. Hydrogen-based microbial ecosystems in the Earth. Science 272:896.
Mackenzie FT, Stoffym M, Wollast R. 1978. Aluminum in seawater: Control by biological activity. Science 199:680–682.
MacLeod RA. 1965. The question of the existence of specific marine bacteria. Bacteriol Rev 29:9–23.
Madsen EL. 1996. Technical comment. Hydrogen-based microbial ecosystems in the Earth. Science 272:896.
Madsen EL, Bollag JM. 1989. Aerobic and anaerobic microbial activity in deep subsurface sediments from the Savannah River Plant. Geomicrobiol J 7:93–101.
Marine Chemistry. 1971. A Report to the Marine Chemistry Panel of the Committee of Oceanography. Washington, DC: Nat Acad Sci.
Marquis RE. 1982. Microbial barobiology. BioScience 32:267–271.
Martinez JS, Zhang GP, Holt PD, Jung H-T, Carrano CJ, Haygood MG, Butler A. 2000. Self-assembling amphiphilic siderophores from marine bacteria. Science 287:1245–1247.
Massana R, DeLong EF, Pedrós-Alió C. 2000. A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl Environ Microbiol 66:1777–1787.
Menzel DW, Rhyter JH. 1970. Distribution and cycling of organic matter in the ocean. In: Wood DE, ed. Organic Matter in Natural Waters. Inst Marine Sci Occasional Publ No.1. Fairbanks, AK: Univ Alaska, pp 31–54.
Minshall GW. 1978. Autotrophy in stream ecosystems. BioScience 28:767–771.
Morita RY. 1967. Effects of hydrostatic pressure on marine bacteria. Oceanogr Mar Biol Annu Rev 5:187–203.
Morita RY. 1975. Psychrophilic bacteria. Bacteriol Rev 39:144–167.
Morita RY. 1980. Microbial life in the deep sea. Can J Microbiol 26:1375–1385.
Nissenbaum A. 1979. Life in the Dead Sea—Fables, allegories, and scientific research. BioScience 29:153–157.
Overmann J, Cypionka H, Pfennig N. 1992. An extremely low light-adapted phototrophic sulfur bacterium from the Black Sea. Limnol Oceanogr 37:150–155.

Park PK. 1968. Seawater hydrogen-ion concentration: Vertical distribution. Science 162:357–358.

Parks RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, Fry JC, Weightman AJ, Harvey SM. 1994. Deep bacterial biosphere in Pacific Ocean sediments. Nature (Lond.) 371:410–413.

Paull CK, Hecker B, Commeau R, Freeman-Lynde RP, Neumann C, Corso WP, Golubic S, Hook JE, Sikes E, Curray J. 1984. Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. Science 226:965–967.

Phelps TJ, Raione EG, White DC, Fliermans CB. 1989. Microbial activities in deep subsurface environments. Geomicrobiol J 7:79–91.

Pope DH, Berger LR. 1973. Inhibition of metabolism by hydrostatic pressure: What limits microbial growth? Arch Mikrobiol 93:367–370.

Reid GK. 1961. Ecology of Inland Waters and Estuaries. New York: Reinhold.

Rheinheimer G. 1980. Aquatic Microbiology. 2nd ed. Chichester, UK: Wiley.

Richardson PL. 1993. Tracking ocean eddies. Am Sci 81:261–271.

Ring Group. 1981. Gulf-Stream cold-core rings: Their physics, chemistry, and biology. Science 212:1091–1100.

Rittenberg SC. 1940. Bacteriological analysis of some long cores of marine sediments. J Mar Res 3:191–201.

Russell BF, Phelps TJ, Griffin WT, Sargent KA. 1992. Procedures for sampling deep subsurface microbial communities in unconsolidated sediments. Ground Water Monitor Rev 12:96–104.

Sargent KA, Fliermans CB. 1989. Geology and hydrology of the deep subsurface microbiology sampling sites at the Savannah River Plant, South Carolina. Geomicrobiol J 7:3–13.

Schwartz JR, Colwell RR. 1976. Microbial activities under deep-ocean conditions. Dev Indust Microbiol 17:299–310.

Seyfried WE Jr, Janecky DR. 19985. Heavy metal and sulfur transport during subcritical hydrothermal alteration of basalt: Influence of fluid pressure and basalt composition and crystallinity. Geochim Cosmochim Acta 49:2545–2560.

Shanks WC III, Bischoff JL, Rosenbauer RJ. 1981. Seawater sulfate reduction and sulfur isotope fractionation in basaltic systems: Interaction of seawater with fayalite and magnetite at 200–350°C. Geochim Cosmochim Acta 45:1977–1995.

Sieburth J McN. 1975. Microbial Seascapes. A Pictorial Essay on Marine Microorganisms and Their Environments. Baltimore, MD: Univ Park Press.

Sieburth J McN. 1976. Bacterial substrates and productivity in marine ecosystems. Annu Rev Ecol Syst 7:259–285.

Sieburth J McN. 1979. Sea Microbes. New York: Oxford Univ Press.

Sieburth J McN, Willis P-J, Johnson KM, Burney CM, Lavoie DM, Hinga KR, Caron DA, French FW III, Johnson PW, Davis PG. 1976. Dissolved organic matter and heterotrophic microneuston in the surface microlayers of the North Atlantic. Science 194:1415–1418.

Sinclair JL, Ghiorse WC. 1987. Distribution of protozoa in subsurface sediments of a pristine groundwater study site in Oklahoma. Appl Environ Microbiol 53:1157–1163.

Sinclair JL, Ghiorse WC. 1989. Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. Geomicrobiol J 7:15–31.
Skinner BJ, Porter SC, Botkin DB. 1999. The Blue Planet. An Introduction to Earth System Science. 2nd ed. New York: Wiley.
Smith FGW, ed. 1974. Handbook of Marine Science, Vol. 1. Cleveland, OH; CRC Press, p 617.
Smith KL, Teal JM. 1973. Deep-sea benthic community respiration: An in situ study at 1850 meters. Science 179:282–283.
Smith RL. 1968. Upwelling. Oceanogr Marine Biol Annu Rev 6:11–46.
Smith W, Pope D, Landau JV. 1975. Role of bacterial ribosome subunits in barotolerance. J Bacteriol 124:582–584.
Stanley SM. 1985. Earth and Life Through Time. New York: WH Freeman.
Stein JL. 1984. Subtidal gastropods consume sulfur-oxidizing bacteria: Evidence from coastal hydrothermal vents. Science 223:696–698.
Stevens TO. 1997. Lithoautotrophy in the subsurface. FEMS Microbiol Rev 20:327–337.
Stevens TO, McKinley JP. 1995. Lithoautotrophic microbial ecosystems in deep basalt aquifers. Science 270:450–454.
Stevens TO, McKinley JP. 1996: Response to technical comment. Hydrogen-based microbial ecosystems in the Earth. Science 272:896–897.
Strahler AN, Strahler AH. 1974. Introduction to Environmental Science. Santa Barbara, CA: Hamilton.
Tortell PD, Maldonado MT, Granger J, Price NM. 1999. Marine bacteria and biogeochemical cycling of iron in the oceans. FEMS Microbiol Ecol 29:1–11.
Tunnicliffe V. 1992. Hydrothermal-vent communities of the deep sea. Am Sci 80:336–349.
Turner JT, Ferrante JG. 1979. Zooplankton fecal pellets in aquatic ecosystems. BioScience 29:670–677.
Turner RD. 1973. Wood-boring bivalves, opportunistic species in the deep-sea. Science 180:1377–1379.
van der Leeden F, Troise FL, Todd DK. 1990. The Water Encyclopedia. 2nd ed. Chelsea, MI: Lewis.
Wangersky PJ. 1976. The surface film as a physical environment. Annu Rev Ecol Syst 7:161–176.
Welch PH. 1952. Limnology. 2nd ed. New York: McGraw-Hill.
Williams J. 1962. Oceanography. Boston, MA: Little, Brown.
Wilson JT, McNabb JF, Balkwill DL, Ghiorse WC. 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground Water 21:134–142.
Wirsen CO, Jannasch HW. 1974. Microbial transformation of some ^{14}C-labeled substrates in coastal water and sediment. Microb Ecol 1:25–37.
Wood EJF. 1965. Marine Microbial Ecology. New York: Reinhold.
Wu J, Sunda W, Boyle EA, Karl DM. 2000. Phosphate depletion in the western North Atlantic Ocean. Science 289:759–762.
Yayanos AA, Dietz AS, van Boxtel R. 1979. Isolation of a deep-sea barophilic bacterium and some of its growth characteristics. Science 205:808–810.
Yayanos AA, Dietz AS, van Boxtel R. 1981. Obligately barophilic bacterium from the Marianas Trench. Proc Natl Acad Sci USA 78:5212–5215.

Zehr JP. 1992. Molecular biology of nitrogen fixation in natural populations of marine cyanobacteria. In: Carpenter EJ, Capone DG, Rueter JG, eds. Marine Pelagic Cyanobacteria: *Trichodesmium* and Other Diazotrophs. Dordrecht: Kluwer Academic; pp 249–264.

ZoBell CE. 1942. Bacteria in the marine world. Sci Monthly 55:320–330.

ZoBell CE. 1946. Marine Microbiology. Waltham, MA: Chronica Botanica.

ZoBell CE, Morita RY. 1957. Barophilic bacteria in some deep sea samples. J Bacteriol 73:563–568.

6

Geomicrobial Processes: A Physiological and Biochemical Overview

6.1 TYPES OF GEOMICROBIAL AGENTS

Various microorganisms, including prokaryotes and eukaryotes, play an active role in certain geological processes, a fact that until fairly recently seems not always to have been sufficiently appreciated by some microbiologists and geologists. Prokaryotic microorganisms include all types of bacteria, and eukaryotic microorganisms include algae, fungi, protozoa and slime molds. Geomicrobially active prokaryotes include members of the domain **Archaea** (formerly known as Archaebacteria and Archaeobacteria) and the domain **Bacteria** (formerly designated as Eubacteria). Both Archaea and Bacteria are prokaryotes because they lack a true nucleus. Each has its genetic information encoded in a large circular polymeric molecule of **deoxyribonucleic acid** (DNA). This structure is often called the bacterial chromosome but unlike the chromosomes of eukaryotic cells, it is not surrounded by a nuclear membrane. The molecular size of a prokaryotic chromosome measures on the order of 10^9 Daltons (Da). Some genetic information in prokaryotes may also be located in one or more extrachromosomal circularized DNA molecules, called **plasmids**. The exact molecular sizes of different plasmids vary, depending on the amount of genetic information they carry, but generally range around 10^7 Da.

Archaea and Bacteria are also classified as prokaryotes because they lack *mitochondria*, membranous organelles that carry out respiration in eukaryotic

cells, and **chloroplasts**, membranous organelles that carry out photosynthesis in eukaryotic cells. In prokaryotes, respiratory activity is carried out in the plasma membrane and photosynthetic activity is carried out either by internal membranes derived from the plasma membrane (purple bacteria, cyanobacteria) or by special internal membranes (green bacteria).

Archaea are not distinguishable from Bacteria when seen as intact cells on a microscopic level. On a submicroscopic level, Archaea exhibit distinct differences from Bacteria in the structure and composition of their cell wall and plasma membrane and in the structure of their ribosomes, which are the sites of protein synthesis. They also differ in key enzymes involved in nucleic acid and protein synthesis (Atlas, 1997; Brock and Madigan, 1988).

Eukaryotic cells differ from prokaryotic cells in possessing a *true nucleus*, which is an organelle enclosed in a double membrane in which the chromosomes and the nucleolus [center for ribosomal ribonucleic acid (RNA) synthesis] are located. Eukarkyotic cells also feature mitochondria, chloroplasts, and vacuoles, all membrane-bound organelles. The structure and operation of their *flagella*, organelles of locomotion, if present, also differ from those of prokaryotic flagella. In eukaryotic cells, some key metabolic processes are highly compartmentalized, unlike those in prokaryotes. Figure 6.1 shows the phylogenetic interrelationships of the prokaryotic domains of Bacteria and Archaea and the Eukaryotes.

Examples of geomicrobially important Archaea include **methanogens** (methane-forming bacteria), *extreme halophiles*, and *thermoacidophiles*. Examples of geomicrobially important Bacteria include some aerobic and anaerobic hydrogen-metabolizing bacteria, iron-oxidizing and -reducing bacteria, manganese-oxidizing and -reducing bacteria, nitrifying and denitrifying bacteria,

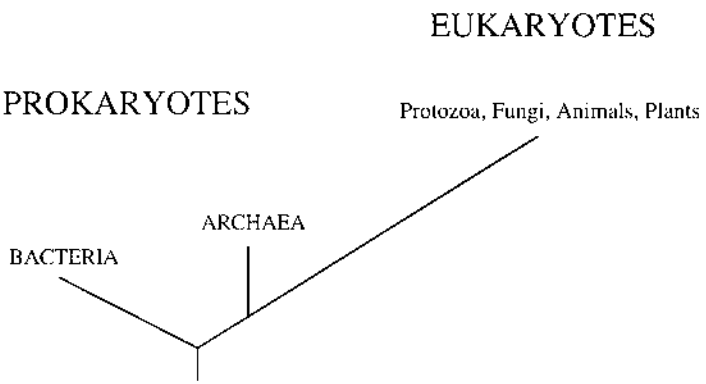


FIG. 6.1 Phylogenetic relationships of the Prokaryotes, domains of the Bacteria and Archaea, and the Eukaryotes, based on the proposal by Woese et al. (1990).

sulfate-reducing bacteria, sulfur-oxidizing and -reducing bacteria, anaerobic photosynthetic sulfur bacteria, oxygen-producing cyanobacteria, and many others. Examples of geomicrobially important eukaryotes include fungi, which can attack silicate, carbonate, and phosphate minerals, among others. They are also important in initiating degradation of somewhat recalcitrant natural organic polymers such as lignin, cellulose, and chitin, as in the O and A horizons of soil (see Chap. 4), or on and in surface sediments. Other geomicrobially important eukaryotes are algae, which together with the cyanobacteria (prokaryotes) are the major source of oxygen in the atmosphere. Some algae promote calcium carbonate precipitation or dissolution, and others precipitate silica as frustules. Still other geomicrobially important eukaryotes include protozoa, some of which lay down siliceous, calcium carbonate, strontium sulfate, or manganese oxide tests, and others may accumulate preformed iron oxide on their cell surface.

6.2 GEOMICROBIALLY IMPORTANT PHYSIOLOGICAL GROUPS OF PROKARYOTES

Prokaryotes can be divided into various physiological groups such as chemolithotrophs, photolithotrophs, mixotrophs, photoheterotrophs, and heterotrophs (Fig. 6.2). Each of these groups includes some geomicrobially important organisms. **Chemolithotrophs** (chemosynthetic autotrophs) include members of both the Bacteria and the Archaea. They are microorganisms that derive energy for doing metabolic work from the oxidation of inorganic compounds and that assimilate carbon as CO_2, HCO_3^-, or CO_3^{2-} (see, e.g., Wood, 1988). **Photolithotrophs** (photosynthetic autotrophs) include a variety of the Bacteria but no known Archaea. They are microorganisms that derive energy for doing metabolic work by converting radiant energy from the sun into chemical energy and that assimilate carbon as CO_2, HCO_3^-, or CO_3^{2-} (photosynthesis). Some of these microbes are anoxygenic (do not produce oxygen from photosynthesis), whereas others are oxygenic (produce oxygen from photosynthesis). **Mixotrophs** include some members of the Bacteria and the Archaea. They may derive energy simultaneously from the oxidation of reduced carbon compounds and oxidizable inorganic compounds, or they may derive their carbon simultaneously from organic carbon and CO_2, or they may derive their energy totally from the oxidation of an inorganic compound but their carbon from organic compounds. **Photoheterotrophs** include mostly Bacteria but also a few Archaea (extreme halophiles). They derive all or part of their energy from sunlight but derive carbon by assimilating organic carbon. **Heterotrophs** include members of both the Bacteria and Archaea. They derive their energy from the oxidation of organic compounds and most or all of their carbon from the assimilation of organic compounds. They may respire (oxidize their energy source) aerobically or

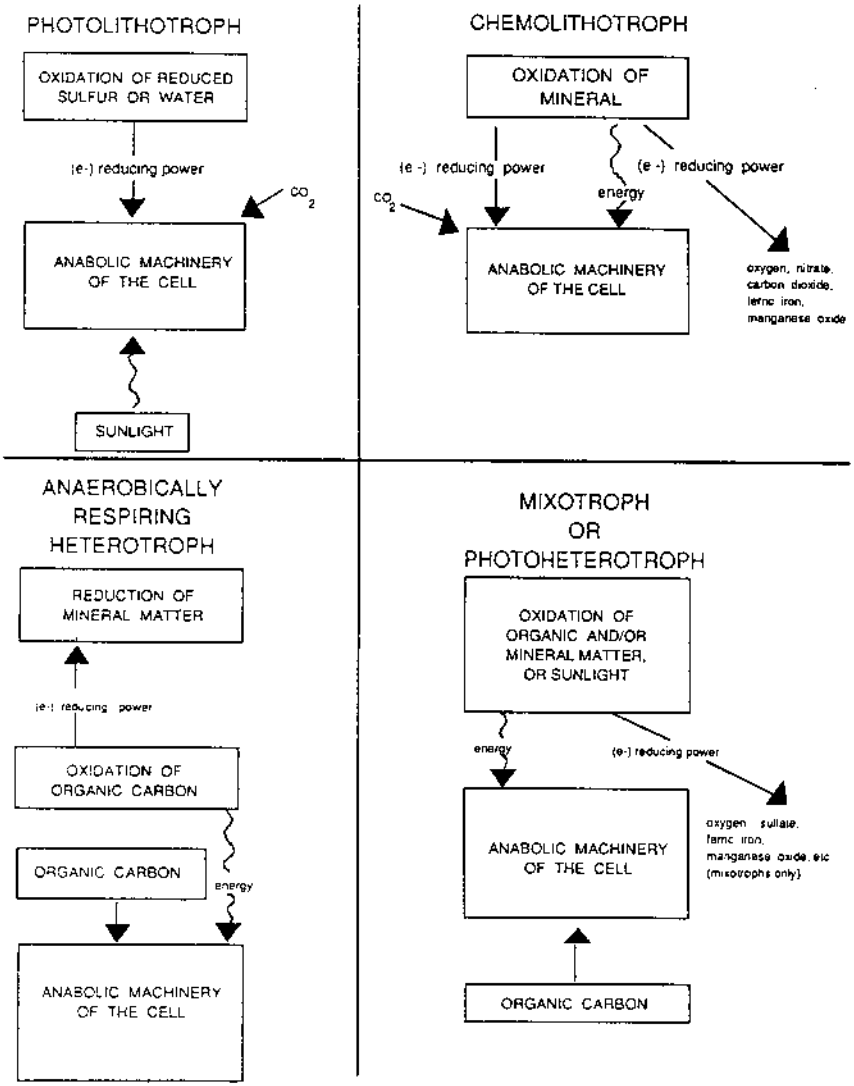


FIG. 6.2 Geomicrobially important physiological groups among prokaryotes.

anaerobically, or they may ferment their energy source by disproportionation (see later discussion of catabolic reactions in Section 6.5).

6.3 ROLE OF MICROBES IN INORGANIC CONVERSIONS IN THE LITHOSPHERE AND HYDROSPHERE

A number of microbes in the biosphere can be considered to be *geological agents*. They may serve as agents of concentration, dispersion, or fractionation of matter. As *agents of concentration*, they cause localized accumulation of inorganic matter by (1) depositing inorganic products of metabolism in or on special parts of the cell, (2) passive accumulation involving surface adsorption or ion exchange, or (3) promoting precipitation of insoluble compounds external to the cell (Ehrlich, 1999). An example of mineral accumulation of an inorganic metabolic product inside a cell is the deposition of polyphosphate (volutin) in the cytoplasm by Bacteria such as *Spirillum volutans*, lactobacilli, rhizobia, and some others. An example of product accumulation in the bacterial cell envelope is the deposition of elemental sulfur granules in the periplasm (region between the plasma membrane and outer membrane of gram-negative bacteria) by *Beggiatoa* and *Thiothrix* (Strohl et al., 1981; Smith and Strohl, 1991). An example of product accumulation at the cell surface is the formation of silica frustules by diatoms (algae), the frustules being their cell walls (de Vrind-de Jong and de Vrind, 1997) (see also Chap. 9).

Examples of passive accumulation of inorganic matter by adsorption or ion exchange are the binding of metallic cations by carboxyl groups of peptidoglycan or phosphate groups of teichoic or teichuronic acids of gram-positive bacteria (e.g., *Bacillus subtilis*) or by the lipopolysaccharide phosphoryl groups of membranes of gram-negative bacteria (e.g., *Escherichia coli*). The bound cations may subsequently react with certain anions, such as sulfide, carbonate, or phosphate, and form insoluble salts that may serve as nuclei in the formation of corresponding minerals (Beveridge et al., 1983; Beveridge, 1989; Beveridge and Doyle, 1989; Doyle, 1989; Ferris, 1989; Geesey and Jang, 1989).

An example of extracellular accumulation is the precipitation of metal cations in the cellular surround (bulk phase) by sulfide produced in sulfate reduction by sulfate-reducing bacteria. Many such sulfides are very insoluble and fairly stable in the absence of oxygen (anoxic conditions) (see Chap. 19).

As *agents of dispersion*, microbes promote dissolution of insoluble mineral matter as, for example, in the dissolution of $CaCO_3$ by respiratory CO_2 (see Chap. 8) or in the biochemical reduction of insoluble ferric oxide or manganese dioxide to soluble compounds (see Chaps. 15 and 16).

As *agents of fractionation*, microbes may act on a mixture of inorganic compounds by promoting selective chemical change involving one or a few compounds of the mixture. One example is the oxidation of arsenopyrite (FeAsS) by *Thiobacillus ferrooxidans* (Ehrlich, 1964) (see also Chap. 13), in which some of the iron solubilized by oxidation reacts with solubilized arsenic from the mineral to precipitate as ferric arsenate. Another example is the preferential solubilization by reduction of Mn(IV) over Fe(III) in ferromanganese nodules by bacteria (Ehrlich et al., 1973, Ehrlich, 2000) (see also Chap. 16). Microbes may also cause fractionation by attacking a stable light isotope of an element in a compound in preference to a heavier isotope of the same element. Examples are the reduction of $^{32}SO_4^{2-}$ in preference to $^{34}SO_4^{2-}$ by some sulfate-reducing bacteria and the assimilation of $^{12}CO_2$ in preference to $^{13}CO_2$ by some autotrophs, both under conditions of slow growth (see discussion by Doetsch and Cook, 1973). Other isotopes that may be separated (fractionated) by microbes include hydrogen from deuterium (Estep and Hoering, 1980), ^{6}Li from ^{7}Li (Sakaguchi and Tomita, 2000), ^{14}N from ^{15}N (Wada and Hattori, 1978), ^{16}O from ^{18}O (Duplessy et al., 1981), ^{28}Si from ^{30}Si (De La Rocha et al., 1997), and ^{54}Fe from ^{56}Fe (Beard et al., 1999). In the laboratory, the magnitude of these fractionations may be relatively large and may involve significant changes in isotopic ratios in a relatively short time. In some natural settings, microbial isotope fractionation can also be of readily detectable magnitude. Studies so far lead to the impression that only a few, mostly unrelated, organisms have the capacity to fractionate stable isotope mixtures.

6.4 TYPES OF MICROBIAL ACTIVITIES INFLUENCING GEOLOGICAL PROCESSES

A number of geological processes at the Earth's surface and in the uppermost crust (deep subsurface) are under the influence of microbes. **Lithification** is a type of geological process in which microbes may produce the cementing substance that binds inorganic sedimentary particles together to form sedimentary rock. The microbially produced cementing substance may be calcium carbonate, iron or aluminum oxide, or silicate. Some types of mineral formation may be the result of microbial activity. Iron sulfides such as pyrite (FeS_2), iron oxides such as magnetite (Fe_3O_4) or goethite (FeOOH), manganese oxides such as vernadite (MnO_2) or psilomelane [$Ba, Mn^{2+}Mn_8^{4+}O_{16}(OH)_4$], calcium carbonates such as calcite and aragonite ($CaCO_3$), and silica (SiO_2) may be generated **authigenically** by microbes (for a more extensive survey, see Lowenstamm, 1981). In some instances, microbes may be responsible for mineral **diagenesis**, in which microbes may cause alteration of rock structure and transformation of primary into secondary minerals, as in the conversion of orthoclase to kaolinite (see

Chap. 4). **Rock weathering** may be promoted by microbes through production of metabolic products, which attack the rock and cause solubilization or diagenesis of some mineral constituents of the rock. Rock weathering may also involve direct enzymatic attack by microbes of certain oxidizable or reducible rock minerals, thereby causing their solubilization or diagenesis. Microbes may contribute to **sediment** accumulation in the form of calcium carbonate tests like those from coccolithophores or foraminifera, silica frustules from diatoms, or silica tests from radiolaria or actinopods in oceans or lakes. The aging of lakes may be influenced by microbes through their rock weathering activity and/or their generation of organic debris from incomplete decomposition of organic matter (see Chap. 5).

Geological processes that are not influenced by microbes include *magmatic activity* or *volcanism*, *rock metamorphism* resulting from heat and pressure, *tectonic activity* related to crustal formation and transformation, and the allied processes of *orogeny* or mountain building. *Wind* and *water erosion* should also be included, although these processes may be facilitated by prior or concurrent microbial weathering activity. Even though microbes do not influence these geological processes, microbes may be influenced by them because these processes may create new environments that may be more or less favorable for microbial growth and activities than before their occurrence.

6.5 MICROBES AS CATALYSTS OF GEOCHEMICAL PROCESSES

Most of the influence that microbes exert on geological processes is physiological. They may act as *catalysts* in some geochemical processes, or they may act as producers or consumers of certain geochemically active substances and thereby influence the rate of a geochemical reaction in which such substances are reactants or products (see Ehrlich, 1996). In either case, they act through their **metabolism**, which has two components. One of these components is **catabolism**, which provides the cell with needed energy through *energy conservation*, and it may also yield to the cell some compounds that can serve as building blocks for polymers. A key reaction in energy conservation is the oxidation of a suitable nutrient or **metabolite** (a compound metabolically derived from a nutrient). The other component of metabolism is **anabolism**. It deals with assimilation (synthesis, polymerization) and leads to the formation of organic polymers such as nucleic acids, proteins, polysaccharides, lipids, and others. It also deals with the synthesis of "inorganic polymers" such as the polysilicate in diatom frustules and radiolarian tests and the polyphosphate granules that are formed by some bacteria and yeasts as energy storage compounds within their cells. Anabolism, by contributing to an increase in cellular mass and duplication

of vital molecules, makes growth and reproduction possible. Catabolism and anabolism are linked to each other in that catabolism provides the energy and some of the building blocks that make anabolism, which is an energy-requiring process, possible. Both catabolism and anabolism may play a geomicrobial role. Catabolism is involved, for instance, in large-scale iron, manganese, and sulfur oxidation. Anabolism is involved, for instance, in the formation of organic compounds from which fossil fuels (peat, coal, and petroleum) are generated. It is also the process by which the diatom frustules and radiolarian tests that accumulate in siliceous oozes are formed.

Catabolism may take the form of aerobic respiration, anaerobic respiration, or fermentation. Catabolism may thus be carried on in the presence or absence of oxygen in air. The role of oxygen is that of *terminal electron acceptor*. Indeed, microorganisms can be grouped as **aerobes** (oxygen-requiring organisms), **anaerobes** (oxygen-shunning microbes), **microaerophilic organisms** (requiring low concentrations of oxygen), or **facultative organisms**, which can adapt their catabolism to operate in the presence or absence of oxygen in air. Facultative organisms use oxygen as a terminal electron acceptor when it is available. When oxygen is not available, they use a reducible inorganic (e.g., nitrate or ferric iron) or organic (e.g., fumarate) compound as a substitute, or they ferment.

Catabolic Reactions: Aerobic Respiration

In *aerobic respiration*, reducing power in the form of hydrogen atoms or electrons is removed in the oxidation of organic or inorganic compounds and conveyed by an *electron transport system* to oxygen to form water. An important metabolic process for generating reducing power is the Krebs tricarboxylic acid cycle (Fig. 6.3) whereby organic substrates are completely oxidized to CO_2 and H_2O (Stryer, 1995). In this process, acetyl ~SCoA produced in the oxidative degradation of a large variety of organic nutrients is enzymatically coupled with oxaloacetate to form citrate with the release of CoASH. The citrate is then converted stepwise to isocitrate, α-ketoglutarate, succinate, fumarate, malate, and back to oxalate. One turn of the cycle produces four hydrogen pairs and two CO_2 as well as one ATP (adenosine 5′-triphosphate) by **substrate-level phosphorylation**. The hydrogen pairs are the source of the reducing power that is fed into the electron transport system and transported to oxygen in aerobic respiration to form water. In the transfer of the reducing power via the electron transport system, some of the energy that is liberated is conserved in special phosphate anhydride bonds of ATP by a chemiosmotic process called **oxidative phosphorylation** (see below). Upon hydrolysis, these bonds (Fig. 6.4) yield 7.3 kcal (30.5 kJ) of free energy per mole at pH 7.0 and 25°C (Stryer, 1995), as opposed to ordinary phosphate ester bonds, which release only about 2 kcal mol^{-1} (8.4 kJ mol^{-1}) of energy under these

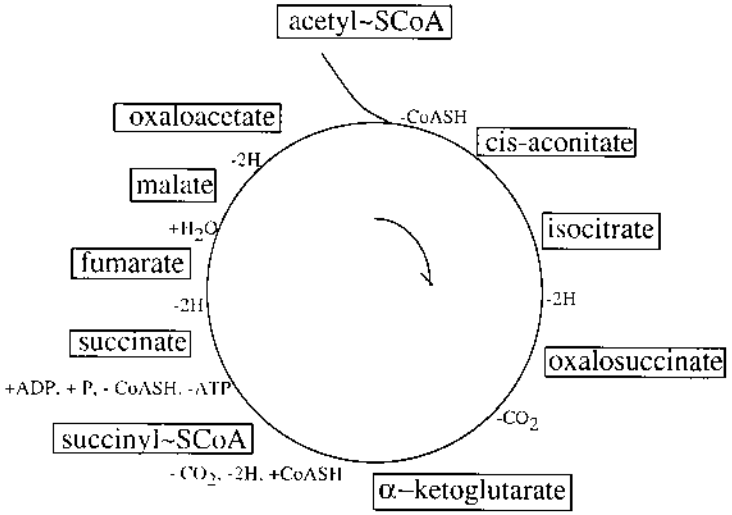
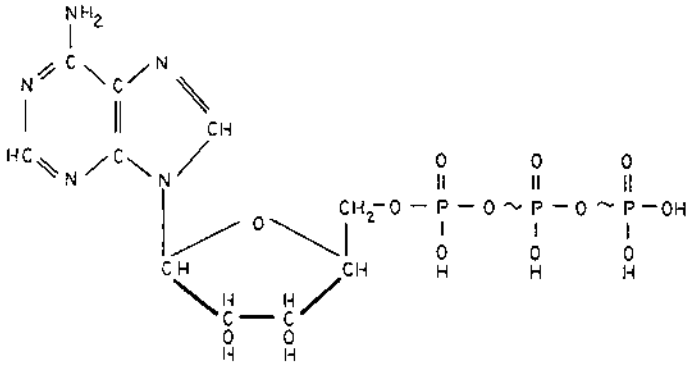


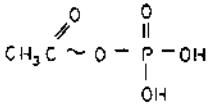
FIG. 6.3 Krebs tricarboxylic acid cycle. One turn of the cycle converts one molecule of acetate to two molecules of CO_2 and from hydrogen pairs (2H), with the formation of one molecule of ATP by substrate phosphorylation. An additional 11 ATP can be formed when the four hydrogen pairs are oxidized to H_2O with oxygen as terminal electron acceptor.

conditions. The energy in high-energy bonds is used by cells for driving energy-consuming reactions, such as syntheses or polymerizations, forward.

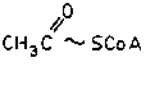
Typical components of the electron transport system include nicotinamide adenine dinucleotide (NAD), flavoproteins (FP), iron-sulfur protein (Fe-S), quinone (CoQ), cytochromes (cyt Fe), and cytochrome oxidase (cyt oxid). They are arranged in complexes in the plasma membrane of some bacteria [e.g., *Paracoccus denitrificans* (Payne et al., 1987; Onishi et al., 1987); marine bacterial strain SSW_{22} (Graham, 1987)] (Fig. 6.5) and in the inner mitochondrial membrane in eukaryotes. The types of electron carriers and enzymes and their arrangement in complexes, if any, differ in different kinds of bacteria. Indeed, in the same bacterium, the carriers may vary quantitatively and/or qualitatively, depending on growth conditions. Whatever the make-up of the assemblage of electron carriers, they interact in a specific sequence such as the one shown in Figure 6.6. Hydrogen or electrons enter the electron transport system where the E_h of the half-reaction by which they are removed from a substrate is near or below the E_h of the appropriate hydrogen- or electron-accepting component of the system. For example, the electrons from the oxidation of H_2 or pyruvate may enter the transport system at the level of complex I via NAD^+ as carrier and are transferred to complex III via CoQ and thence to complex IV via cytochrome c.



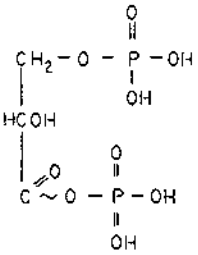
ADENOSINE – 5' – TRIPHOSPHATE (ATP)



ACETYL PHOSPHATE



ACETYL – COENZYME A



1,3 – DIPHOSPHOGLYCERIC ACID

FIG. 6.4 Examples of compounds containing one or more high-energy phosphate bonds (~).

Complex IV transfers the electrons it receives to O_2, which is then transformed to H_2O (Stryer, 1995). Electrons from the oxidation of succinate enter the transport system via complex II and are then transferred to complex III and so on to O_2. Electrons from the oxidation of ferrous iron enter the electron transport system at the level of complex IV. Table 6.1 lists the E_h values for some geomicrobially

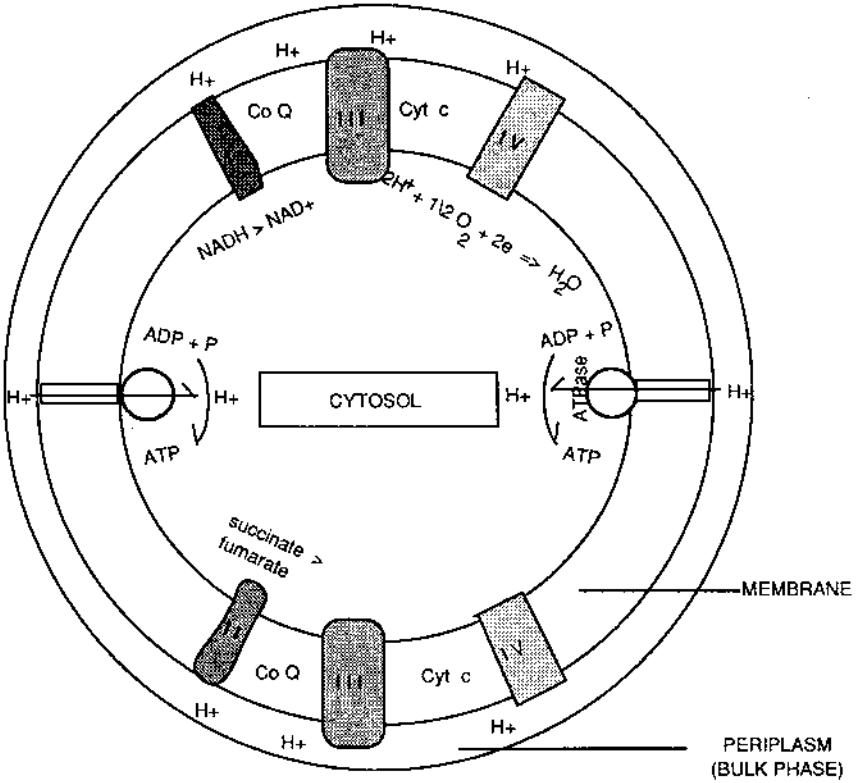


FIG. 6.5 Schematic displays of the bioenergetic machinery in a prokaryotic (domain Bacteria) cell envelope. Structures labeled I, II, III, and IV represent specific electron transport complexes involved in some prokaryotes. Complex I, reactive with NADH, includes a flavoprotein and Fe-S protein; complex II, reactive with succinate, includes succinic dehydrogenase (another flavoprotein) and Fe-S protein; complex III includes cytochromes b and c_1 and an Fe-S protein; complex IV includes cytochrome oxidase (e.g., cytochrome $a + a_3$). Coenzyme Q (CoQ) and cytochrome c (cyt c) shuttle electrons between respective complexes. Proton translocation from the cytosol to the periplasm involves complexes I or II, CoQ + complex III, and often complex IV. Oxygen reduction to water occurs on the inner surface of the plasma membrane. ATPase is the site of ATP synthesis.

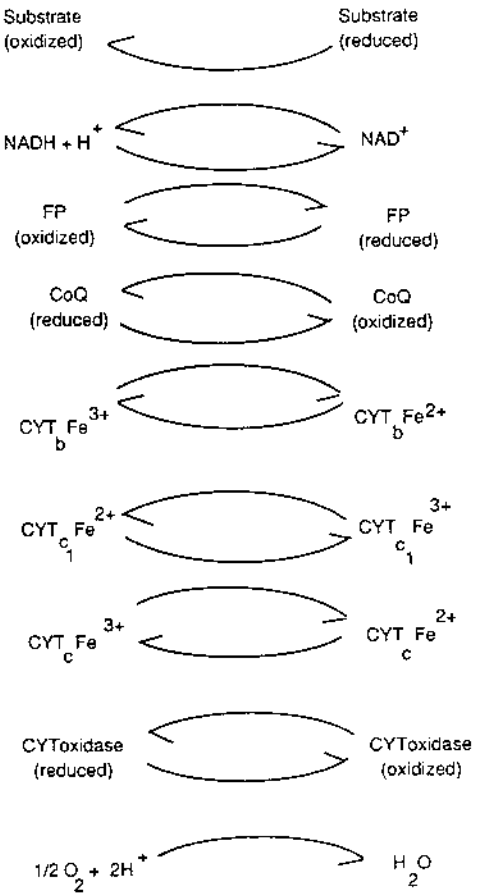


FIG. 6.6 Schematic representation of the sequence of interactions of components of an electron transport system in a bacterial membrane by which reducing power is transferred from a substrate to oxygen.

important enzyme-catalyzed oxidations, the level at which their hydrogens or electrons are probably fed into the electron transport system upon their oxidation, and also the maximum number of high-energy phosphate bonds (ATP) that may be generated in the transfer of hydrogen or electron pairs to oxygen.

It is important to recognize that in prokaryotic cells the electron transport system is located in the plasma membrane (Fig. 6.7A), whereas in eukaryotic cells it is located internally in special organelles called mitochondria (Fig. 6.7B).

TABLE 6.1 Microbially Catalyzed Oxidations of Geological Significance: Some Characteristics of Their Interaction with the Electron Transport System

Reaction	E'_h at pH 7.0 (V)	Entrance level into electron transport system	ATP/$2e^-$ or 2H
$Fe^{2+} \rightarrow Fe^{3+} + e^-$	+0.77	Complex IV	1
$S^0 + 4H_2O \rightarrow SO_4^{2-} + 8H^+ + 6e^-$	−0.20	Complex III or IV	2 or 1[a]
$H_2S \rightarrow S^0 + 2H^+ + 2e^-$	−0.27	Complex I or III	3 or 2
$H_2 \rightarrow 2H^+ + 2e^-$	−0.42	Complex I or III	3 or 2
$Mn^{2+} + 2H_2O \rightarrow MnO_2 + 4H^+ + 2e^-$	+0.46	Complex IV (?)	1

[a] Add 0.5 mol of ATP per mol of SO_3^{2-} oxidized to SO_4^{2-} if substrate-level phosphorylation is part of the oxidation process.

As a result bacteria endowed with appropriate oxidoreductases (enzymes that transfer hydrogen atoms or electrons) in their cell envelope are able to oxidize or reduce insoluble substrates that cannot be taken into the cell, such as elemental sulfur, iron sulfide, iron oxide, and manganese oxide. Because the essential enzymes are located in the periplasmic space and/or plasma membrane of prokaryotes, or even the outer membrane in the case of at least one gram-negative bacterium (Myers and Myers, 1992), they are able to make direct contact with the substrate. Eukaryotic cells, on the other hand, are unable to carry out such reactions because their electron transport system is located on the inner membrane of mitochondria, which reside in the cytoplasm of these cells. The mitochondrial electron transport system, being removed from the cell surface, thus lacks direct access to insoluble substrates (Ehrlich, 1978).

Catabolic Reactions: Anaerobic Respiration

Whereas in aerobic respiration oxygen is always the terminal electron acceptor, in *anaerobic respiration* other reducible compounds such as nitrate, sulfate, elemental sulfur, carbon dioxide, ferric oxide or some other ferric compounds, and manganese oxides or an organic compound such as fumarate serve as terminal electron acceptors instead. The process is normally associated with some Archaea and some Bacteria including some cyanobacteria. Anaerobic bacteria may be facultative (e.g., nitrate reducers, some iron and manganese reducers), or obligate anaerobes (sulfate-reducing bacteria, methanogens, acetogens, some other iron and manganese reducers). Certain bacterial respirers may reduce O_2 and concurrently another inorganic electron acceptor [certain nitrate, chromate, and MnO_2 reducers (see Chaps. 12, 16, and 17, respectively)]. In most

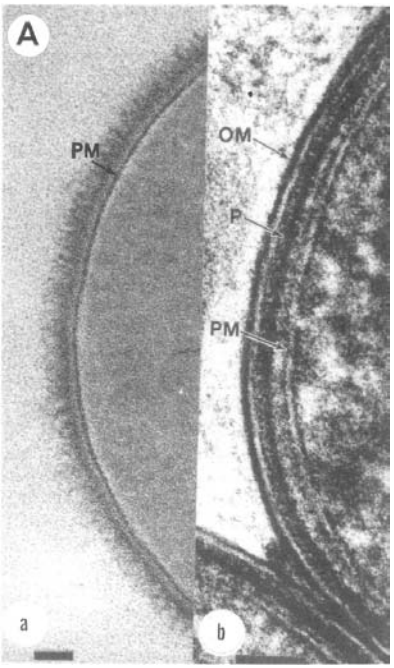


FIG. 6.7A Location of electron transport systems (ETS) in prokaryotic and eukaryotic cells. Thin sections of the gram-positive cell wall of *Bacillus subtilis* (a) and the gram-negative cell wall of *Escherichia coli* (b). Both sections were prepared by freeze substitution. OM, outer membrane; PM, plasma membrane; P, periplasmic gel containing peptidoglycan located between the outer and plasma membranes. In both types of cells, the ETS is located in the plasma membrane. The bars in (a) and (b) equal 25 nm. (From Beveridge, 1989, with permission.)

cases of anaerobic respiration by facultative organisms, oxygen competes with the other possible electron acceptors and thus must be absent or present at significantly lower concentration than in normal air. Anaerobic respiration usually employs some of the hydrogen and electron carriers of aerobic respiration but usually substitutes a suitable terminal reductase for cytochrome oxidase to convey electrons to the terminal electron acceptor that replaces oxygen. If the organic substrate being consumed is oxidized completely, the tricarboxylic acid cycle may be involved, but other pathways may be used instead. Among the best characterized of these systems are those in which sulfate and nitrate are reduced. Like aerobic respiration, anaerobic respiration involves the bacterial plasma membrane.

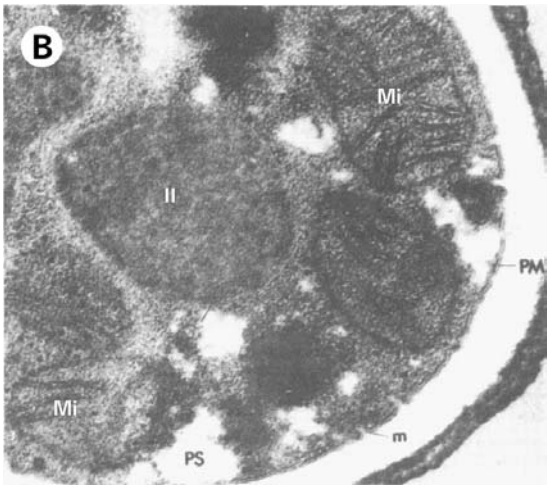


FIG. 6.7B Location of electron transport systems (ETS) in prokaryotic and eukaryotic cells. Cross section of a dormant conidium (spore) of *Aspergillus fumigatus*, a fungus and eukaryote (×64,000). ETS is located in the mitochondria. Mi, mitochondria; PM, plasma membrane; m, thin layer of electron-dense material; PS, polysaccharide storage material; II, membrane-bound storage body. (Courtesy of W. C. Ghiorse.)

Catabolic Reactions: Fermentation

Fermentation is a catabolic process in which energy is conserved in a disproportionation process in which part of the energy-yielding substrate consumed is oxidized by reducing the remainder of the consumed substrate. No externally supplied terminal electron acceptor is involved in this redox process. Glucose fermentation to lactic acid by the Embden–Meyerhoff pathway is a typical example (Fig. 6.8). Pairs of hydrogen atoms are removed in an oxidation step from an intermediate metabolic product, glyceraldehyde 3-phosphate, resulting in formation of 1,3-diphosphoglyceric acid. The hydrogen pairs that are removed are transferred to pyruvate, thereby reducing it to lactic acid. The source of the pyruvate is the enzymatic transformation of 1,3-diphosphoglycerate. A variant of this pathway leads to the formation of ethanol and CO_2. Other mechanisms of glucose fermentation that can lead to the formation of acetate include the Entner–Doudoroff pathway, the pentose phosphate pathway, and the pentose phosphoketolase pathway (Stryer, 1995). Recently a new glycolytic pathway leading to the formation of acetate and formate was discovered in the archeon *Thermococcus zilligii* (Xavier et al., 2000).

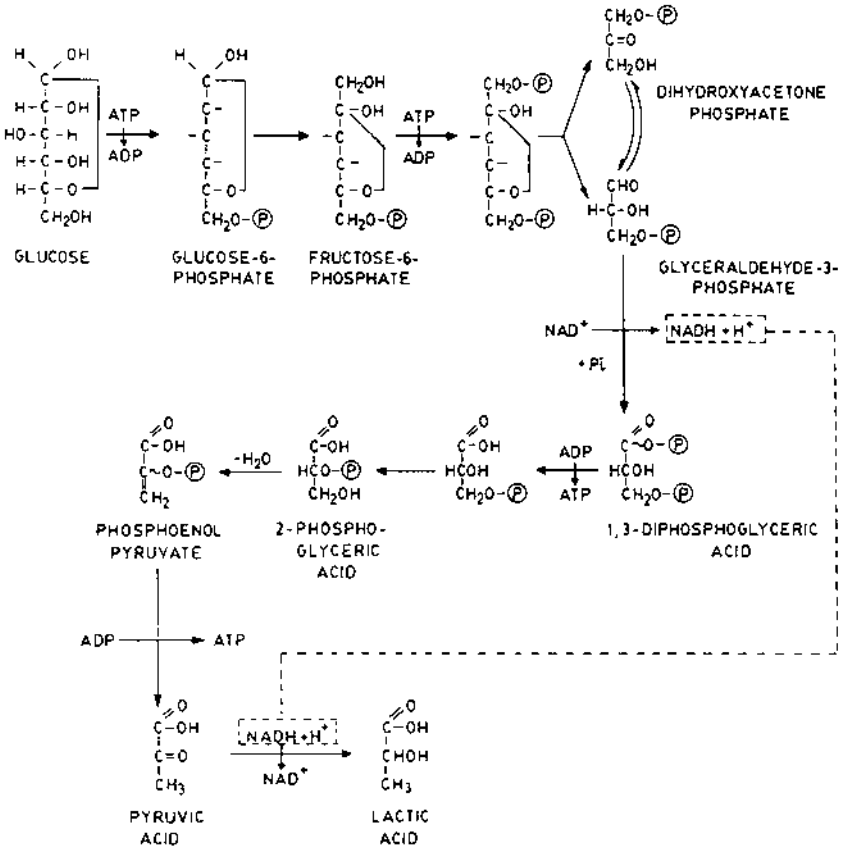


FIG. 6.8 Conversion of glucose to lactic acid by glycolysis, an example of fermentation. Note that in this reaction sequence, biochemically useful energy in the form of ATP is generated exclusively by substrate-level phosphorylation.

Energy released in fermentation that is useful to the cell is conserved by substrate-level phosphorylation (see next section); a membrane-bound electron transport system is almost never involved. Major exceptions are the fermentation of acetate to methane and CO_2 (see Chap. 21) and the anaerobic disproportionation of thiosulfate to sulfide and sulfate (Bak and Cypionka, 1987; Bak and Pfennig, 1987; Finster et al., 1998; Janssen et al. 1996; Jackson and McInerney, 2000) (see Chap. 18). Fermentation always occurs in the cytoplasm of a cell. It is a process of which a number of bacteria, both facultative and anaerobic, are

capable, but it is relatively rare among eukaryotic organisms. Certain fungi, such as the yeast *Saccharomyces cerevisiae*, are exceptions.

How Energy Is Generated by Aerobic and Anaerobic Respirers and by Fermenters During Catabolism

In aerobic and anaerobic respiration, most useful energy is trapped in high-energy phosphate bonds conserved in ATP as a result of **oxidative phosphorylation**. The reaction leading to the formation of a high-energy bond may be summarized as*

$$\mathrm{ADP} + \mathrm{P_i} \rightarrow \mathrm{ATP} \tag{6.1}$$

Reaction (6.1) is energy-consuming and is made possible by charge separation between the inside and outside of the plasma membrane in prokaryotes. In most respiring prokaryotes, the charge separation results from the passage of electrons down the electron transport chain to oxygen, which is coupled concurrently with the pumping of protons across the plasma membrane from the cell interior to the cell periplasm or its equivalent. In eukaryotes, the passsage of electrons down the electron transport chain is coupled to the pumping of protons from the interior (matrix) to the outside of the inner membrane of the mitochondria. The cytoplasm of actively respiring prokaryotic cells or the matrix of active mitochondria of eukaryotic cells is alkaline relative to the outside of the respective membranes enclosing them and therefore more electronegative. The plasma membrane and the inner mitochondrial membrane are impermeable to protons except at the sites where the enzyme complex adenosine 5′-triphosphatase (ATPase) is located. The ATPase permits the re-entry of protons into the prokaryotic cell or the matrix of the mitochondrion through a proton channel (Fig. 6.5). It couples this proton re-entry with ATP synthesis [reaction (6.1)]. Proton reentry via ATPase is facilitated in aerobes by the consumption of protons in the reduction of O_2 to water catalyzed by cytochrome oxidase on the inside of the plasma membrane or inner mitochondrial membrane. In anaerobically respiring bacteria, protons may be consumed in the reduction of the electron acceptor that replaces oxygen, the reduction being catalyzed by an enzyme other than cytochrome oxidase. The energy that drives reaction (6.1) comes from the proton gradient and from the membrane potential,

$$\mathrm{PMF} = \Delta\psi - 2.3RT\,\frac{\Delta\mathrm{pH}}{F} \tag{6.2}$$

In which PMF is the proton motive force, $\Delta\psi$ is the transmembrane potential, ΔpH is the pH gradient across the membrane, R is the universal gas constant, T is absolute temperature, and F is the Faraday constant. The overall process by which

*ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; P_i, inorganic phosphate.

energy is generated in aerobic and anaerobic respiration is called chemiosmosis [see Hinkle and McCarty, (1978) and Stryer (1995) for a further discussion of this process]. A maximum of three molecules of ATP may be formed per electron pair transferred from donor to terminal acceptor in aerobic respiration, and a probable maximum of two in anaerobic respiration.

In methanogens, the electron transport system is as yet incompletely characterized. It is generally agreed, however, that they use a chemiosmotic mechanism for the production of ATP. Many methanogens achieve charge separation in the form of a pH gradient and transmembrane potential by generating protons in the oxidation of H_2 in a periplasmic space equivalent outside the plasma membrane and by conducting electrons to the cell interior for use in CO_2 assimilation. This model is supported by evidence developed by Blaut and Gottschalk (1984), Butsch and Bachofen (1984), Mountford (1978), and Sprott et al. (1985). Because the interior of the cells of methanogens has a pH near neutrality, H_2 oxidation in the periplasmic space equivalent generates a pH gradient in actively metabolizing cells. This pH gradient appears to be utilized in the generation of ATP by ATP synthase (ATPase) complexes projecting from the inside surface of the membrane. The electrons removed in the oxidation of hydrogen are conveyed to the cell interior, probably by a membrane-bound hydrogenase, and used in the reduction of CO_2 to methane (see Chap. 21). The charge separation mechanism of methanogens for generating ATP by oxidative phosphorylation is probably illustrative of the earliest chemiosmotic mechanisms from which the more elaborate systems utilizing a variety of different membrane-bound electron carriers and enzymes found in modern aerobic and anaerobic respirers evolved. Some methanogens appear to use Na^+ rather than H^+ to achieve charge separation (see Chap. 21).

In fermentation, useful energy is formed by **substrate-level phosphorylation**, a process in which a high-energy bond, which traps some of the total free energy released during oxidation, is formed on the substrate that is being oxidized. An example is the oxidation of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate in glucose fermentation, illustrated in Figure 6.8. Substrate-level phosphorylation may also occur during aerobic and anaerobic respiration, but it contributes only a small portion of the total energy conserved in high-energy bonds by cells. Clearly, aerobic and anaerobic respiration are much more efficient energy yielding processes than fermentation. It takes less substrate to satisfy a fixed energy requirement of a cell if a substrate is oxidized by aerobic or anaerobic respiration than when it is oxidized by fermentation. If the energy-yielding substrate is organic, the greater efficiency may also be due to the fact that respirers may oxidize the substrate completely to CO_2 and H_2O whereas fermenters cannot.

Whereas many of the microbes that oxidize inorganic substrates to obtain energy are aerobes, a few are not. All autotrophically growing methanogens

oxidize hydrogen gas (H_2) anaerobically by transferring electrons from H_2 to CO_2 to form methane (CH_4), generating ATP by oxidative phosphorylation in the process. *Homoacetogens* carry out a similar reduction of CO_2 by hydrogen but form acetate instead of methane (Eden and Fuchs, 1983):

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (\Delta G^\circ, -25 \text{ kcal or } -104.8 \text{ kJ}) \quad (6.3)$$

Some oxidizers of sulfur compounds can transfer electrons from a reduced sulfur substrate such as thiosulfate or elemental sulfur to nitrate in the absence of oxygen. In the presence of oxygen, these sulfur-oxidizing organisms transfer the electrons from the reduced sulfur compounds to oxygen. The maximum ATP yield in methane formation from H_2 reduction of CO_2 and in the oxidation of reduced sulfur by nitrate, two examples of anaerobic respiration, has not yet been clearly established.

How Chemolithotrophic Bacteria (Chemosynthetic Autotrophs) Generate Reducing Power for Assimilating CO_2 and Converting It to Organic Carbon

Unlike heterotrophs, most chemolithotrophs when reducing CO_2 with NADPH have a special problem in generating NADPH. These chemolithotrophs, which possess an electron transport chain containing Fe-S proteins, quinones, and cytochromes in their plasma membrane whether they are aerobic or anaerobic respirers, depend on *reverse electron transport* to reduce $NADP^+$ to NADPH. For this reduction, electrons must travel against a redox gradient with the expenditure of energy in the form of ATP. The source of the electrons, which is also the source of energy, usually has a midpoint potential (E_h) that is significantly higher than that of the NADP/NADPH couple. Methanogens, which do not use NADPH for reducing fixed CO_2 to organic carbon but instead employ unique hydrogen carriers such as factor F_{420} (8-OH-deazaflavin) and carbon dioxide reducing factor (CDR), appear not to consume ATP in CO_2 reduction by hydrogen (Ferris, 1993). Homoacetogens are another exception. They employ ferredoxin and other Fe-S proteins, whose reduction does not require the expenditure of energy (Pezacka and Wood, 1984).

How Photosynthetic Microbes Generate Energy and Reducing Power

Anoxygenic bacteria, such as the purple and green bacteria, purple non-sulfur bacteria, and some cyanobacteria that have the capacity to grow anaerobically in the presence of H_2S, generate their ATP by transducing light energy of

appropriate wavelengths into chemical energy, which they conserve in high-energy phosphate bonds via cyclic or noncyclic photophosphorylation (Gottschalk, 1985). They work on a chemiosmotic principle analogous to respiration. In cyclic photophosphorylation, as in purple sulfur bacteria, electrons pass from a reduced, low potential Fe-S protein (E_h − 530 mV) along an electron transport pathway, which includes membrane-bound quinones and cytochromes, to bacteriochlorophyll. High-energy phosphate bonds are generated in this phase of electron passage and conserved in ATP. The passage of the electrons is coupled to proton pumping and a resultant proton gradient as in respiration, except that in this case the proton pumping is in a direction opposite that of respiration, i.e., from the outside of the membrane barrier to the inside. The proton motive force thus generated causes ATPase in the photosynthetic membrane to generate ATP from ADP and P_i. For the electrons to return to the low-potential Fe-S protein from the high-potential bacteriochlorophyll in cyclic photophosphorylation, they have to be energized by light absorption at an appropriate wavelength (Fig. 6.9). It is light, therefore, which drives the cyclic movement of the electrons.

In green sulfur bacteria, photophosphorylation is noncyclic as well as cyclic (Fig. 6.9). The cyclic mechanism is similar to that in the purple sulfur bacteria. In the noncyclic photophosphorylation process, ATP is synthesized in a reaction sequence in which an external electron donor such as H_2S, S^0 or $S_2O_3^{2-}$, for instance, reduces chlorobium chlorophyll. The electrons that have reduced the chlorophyll are then used to reduce $NADP^+$ to NADPH. This requires input of light energy because the midpoint potential for chlorophyll reduction is much higher ($\sim$ +400 mV) than that for $NADP^+$ reduction ($\sim$ −350 mV). As in most known chemolithotrophs, the NADPH is needed for CO_2 assimilation. The ATP-synthesizing mechanisms in both cyclic and noncyclic photophosphorylation of green sulfur bacteria involve chemiosmosis. The extent to which the cyclic and noncyclic mechanisms operate depends on the demands for ATP and NADPH by the cell.

Cyanobacteria also use a noncyclic photophosphorylation process for generating ATP, but they use a more complex pathway than the green sulfur bacteria (Fig. 6.9). Their photosynthetic machinery, which is normally oxygenic because it uses H_2O as a source of reducing power, unlike that of either the purple or green sulfur bacteria, involves two major components, photosystems I and II. These are linked to each other by a series of interacting electron carriers that transfer electrons from photosystem II to photosystem I and promote proton pumping, which permits chemiosmotic ATP synthesis. Photosystem II generates electrons by the photolysis of water with transduced light energy. Photosystem I sends the electrons received from photosystem II to $NADP^+$ to form NADPH with another boost from transduced light energy. As in green sulfur bacteria, NADPH is required for reducing fixed CO_2. Besides the noncyclic photo-

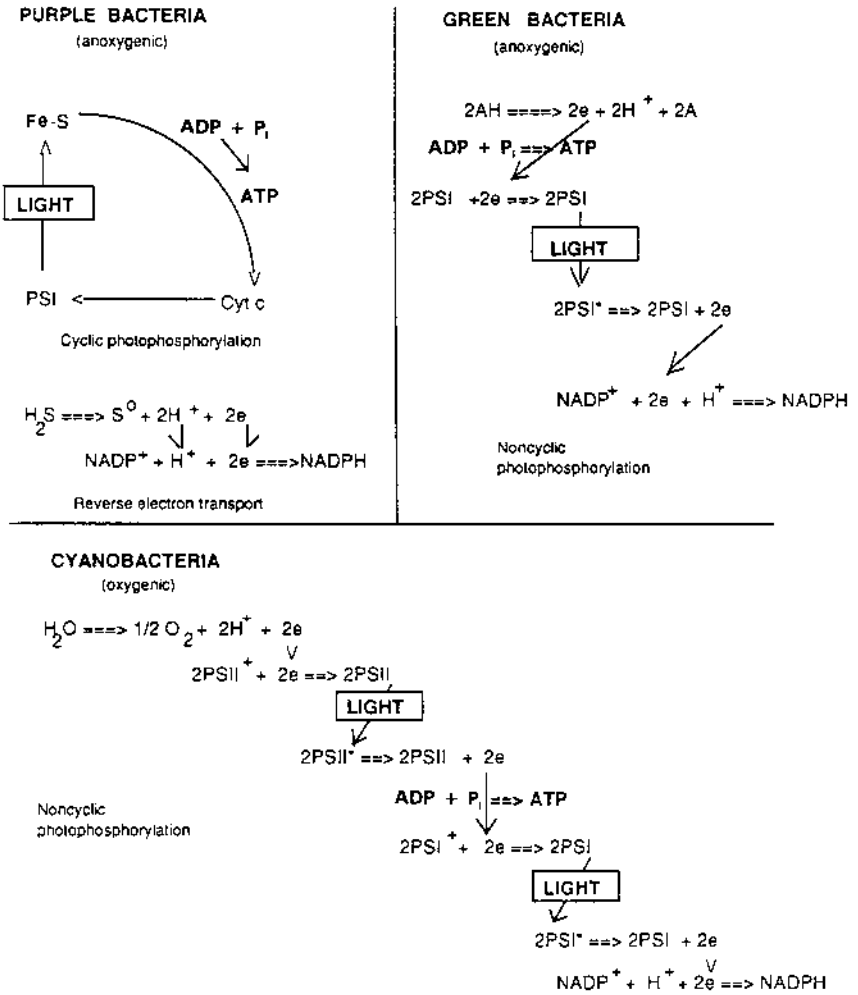


FIG. 6.9 Diagrammatic representation of the mechanisms of photophosphorylation and generation of reducing power (NADPH) in purple and green photosynthetic bacteria and in cyanobacteria. PSI, photosystem I; PSII, photosystem II. (Adapted from Stanier et al., 1986.)

phosphorylation, oxygen-producing cyanobacteria perform cylic photophosphorylation that involves only photosystem I to generate additional ATP.

The need for NADPH that purple sulfur bacteria have to reduce CO_2 to organic carbon is met by reverse electron transport that is not *directly* dependent

on light energy input (Fig. 6.9). They transfer electrons from a high-potential membrane carrier to low-potential membrane carriers and finally to $NADP^+$ by consumption of ATP. The energy consumption is needed because the electrons in this instance travel against a redox gradient. For further discussion of these photosynthetic processes, the reader is referred to Stanier et al. (1986) and to Atlas (1997).

Anabolism: How Energy Trapped in High-Energy Bonds Is Used to Drive Energy-Consuming Reactions

As an example of how aerobic chemolithotrophs couple ATP formation to CO_2 assimilation and reduction to organic carbon, the process in *Thiobacillus ferrooxidans*, an acidophilic iron oxidizer, will be considered. This organism oxidizes ferrous to ferric iron at acid pH,

$$2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^- \tag{6.4}$$

Some of the reducing power (e^-) generated in this way is transferred to oxygen:

$$\tfrac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O \tag{6.5}$$

with the simultaneous chemiosmotic production of ATP,

$$ADP + P_i \rightarrow ATP \tag{6.6}$$

At maximum efficiency, one ATP is formed for every electron pair ($2e^-$) transferred to oxygen. The remaining reducing power from the oxidation of the ferrous iron is used to reduce pyridine nucleotide (NAD^+, $NADP^+$). Because the electrons in this case have to travel against a redox gradient from +800 mV (high-potential Q-cycle intermediate in *T. ferrooxidans* at pH 2) (see Ingledew, 1982; Ehrlich et al., 1991) to −305 mV (E_m at pH 6.5 for $NADP^+$/NADPH), energy stored in high-energy phosphate bonds of ATP has to be consumed,

$$NAD^+ + 2H^+2e^- + 2ATP \rightarrow NADH + H^+ + 2ADP + P_i \tag{6.7}$$

$$NADH + H^+ + NADP^+ \rightarrow NAD^+ + NADPH + H^+ \tag{6.8}$$

The $NADPH + H^+$, together with some ATP, is used in the assimilation of CO_2 and its reduction to organic carbon,

$$\text{Ribulose 5-phosphate} + ATP \rightarrow \text{ribulose 1,5-diphosphate} + ADP \tag{6.9}$$

$$\text{Ribulose1,5-diphosphate} + CO_2 \rightarrow 2(\text{3-phosphoglycerate}) \tag{6.10}$$

$$2(\text{3-phosphoglycerate}) + 2NADPH + 2H^+ + 2ATP \rightarrow 2(\text{glycerealdehyde 3-phosphate}) + 2NADP^+ + 2ADP + 2P_i \tag{6.11}$$

From glyceraldehyde 3-phosphate, the various organic constituents of the cell are then manufactured, including the building blocks for polymers such as proteins, nucleic acids, lipids, and polysaccharides, which are subsequently combined into corresponding polymers with the expenditure of additional ATPs because polymerizations are energy-requiring reactions. Also, some ribulose 5-phosphate is regenerated to permit continued CO_2 fixation [reactions (6.10) and (6.11)]. Although generally chemolithotrophs can grow in the complete absence of organic matter under laboratory conditions, many if not all of these organisms can assimilate some types of organic compounds such as amino acids and vitamins. Some chemolithotrophs are able to use organic carbon as a sole energy source under some conditions (facultative chemolithotrophs), but others cannot (*obligate chemolithotrophs*).

Anaerobic chemolithotrophs, such as methanogens, homoacetogens, and some sulfate reducers, use a different mechanism for assimilation of CO_2. They form acetate (CH_3COO^-) from two molecules of CO_2. This involves the stepwise reduction of one CO_2 to a methyl carbon and of the other to carbon monoxide (CO) and then the formation of a carboxyl carbon. The methyl and carboxyl carbons are then combined to form acetate in an ATP-consuming process. The acetate is then carboxylated by combining with another molecule of CO_2 in another ATP-consuming process to form pyruvate (Fig. 6.10). This product is a key precursor for the formation of all other monomeric building blocks from which the various polymers are formed by ATP-consuming processes.

Anoxygenic photolithotrophs assimilate CO_2 by one of several different mechanisms. Purple sulfur bacteria usually fix CO_2 and reduce it to organic carbon by the same set of reactions, (6.9)–(6.11), as aerobic chemolithotrophs like *T. ferrooxidans*. They obtain the needed NADPH through reduction of $NADP^+$ by reverse electron transport with H_2, H_2S, S^0, or $S_2O_3^{2-}$ as electron donors (see above).

The filamentous green photolithotroph *Chloroflexus aurantiacus* fixes CO_2 by a 3-hydroxypropionate cycle (Ivanovsky et al., 1993; Strauss and Fuchs, 1993;

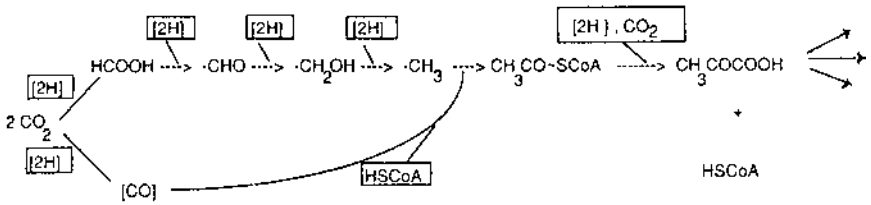


FIG. 6.10 Pathway for carbon assimilation in methanogens [the activated acetate ($CH_3CO \sim SCoA$) pathway]. Pyruvate ($CH_3COCOOH$) is a key intermediate for forming various building blocks for the cell, including sugars, amino acids, fatty acids, and so on.

Eisenreich et al., 1993; Herter et al., 2001). In the cycle as proposed by Herter et al. (2001), CO_2 is incorporated into acetyl~SCoA to form malonyl~SCoA, which in turn is transformed into 3-hydroxypropionate, and from which propionyl~SCoA is formed. The propionyl~SCoA is transformed into succinyl~SCoA via methylmalonyl~SCoA by further CO_2 fixation. The succinyl~SCoA is transformed into malonyl~SCoA, which is then cleaved into acetyl~SCoA and glyoxalate, completing the cycle (Fig. 6.11). How glyoxalate is used in the synthesis of cellular carbon compounds remains unclear at this time according to Herter et al. (2001).

Green sulfur bacteria of the genus *Chlorobium* fix CO_2 and reduce it by a reverse tricarboxylic acid cycle (Fig. 6.12). In this process, CO_2 is combined with pyruvate in an ATP-consuming process to form oxalate, which is then converted via malate, fumarate, and succinate to 2-ketoglutarate, the last step requiring

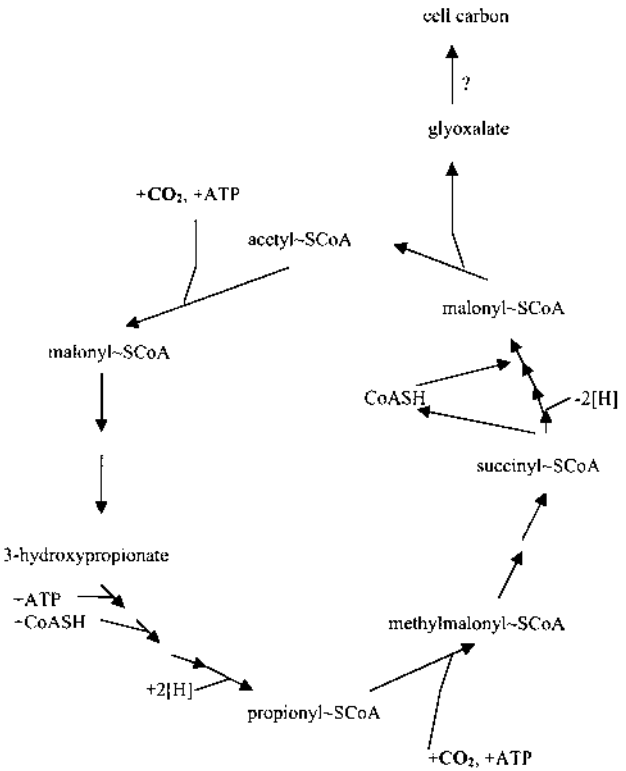


FIG. 6.11 Pathway of autotrophic CO_2 fixation by *Chloroflexus aurantiacus* as proposed by Herter et al. (2001). (Adapted from Herter et al., 2001.)

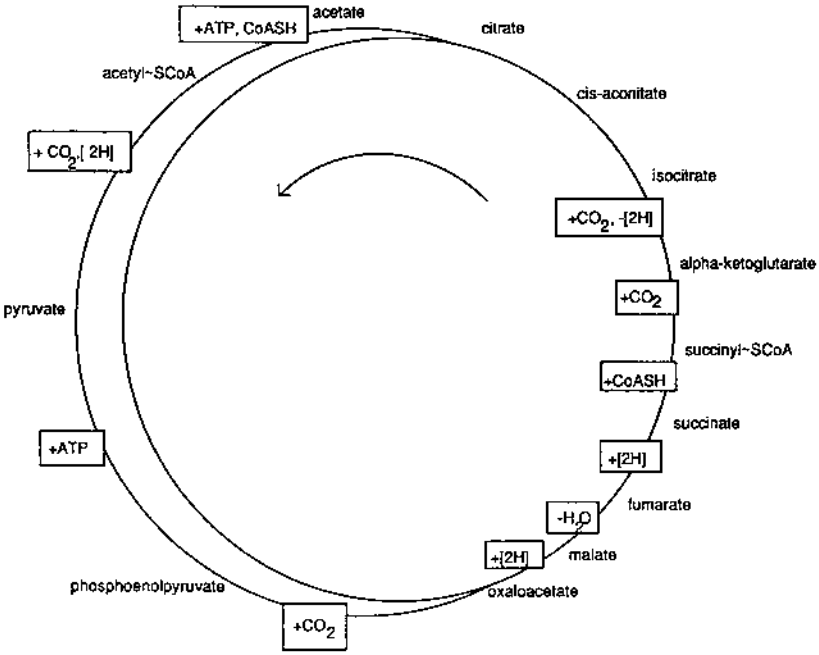


FIG. 6.12 The reverse tricarboxylic acid cycle used by green sulfur bacteria for carbon assimilation. (Modified from Stanier et al., 1986; scheme originally presented by Evans et al., 1966.)

consumption of ATP. The 2-ketoglutarate is a key precursor in amino acid synthesis as well as being a precursor for citrate synthesis. Formation of citrate involves fixation of another CO_2. The citrate is cleaved to oxaloacetate and acetate. The acetate serves as precursor in the synthesis of pyruvate by CO_2 fixation with ATP consumption, thus completing the reverse tricarboxylic acid cycle. The pyruvate is a key precursor for the synthesis of other biochemical building blocks. NADH and NADPH needed in the operation of this cycle are generated by a noncyclic photoreduction mechanism (see above). Although once thought unique to *Chlorobium*, the reverse tricarboxylic acid cycle has since been found to operate as a mechanism of CO_2 assimilation in some chemosynthetic autotrophs, e.g., *Aquifex pyrophilus*, a chemolithotrophic, H_2-oxidizing member of the domain Bacteria, and *Thermoproteus neutrophilus*, a chemolithotrophic, thermophilic, H_2-oxidizing and S^0-reducing archaeon (Beh et al., 1993).

Oxygenic photolithotrophs fix CO_2 and reduce it to organic carbon by a reaction sequence similar to reactions (6.9)–(6.11). They produce NADPH for

this process via noncyclic photophosphorylation and ATP by both noncyclic and cyclic photophosphorylation (see above).

Carbon Assimilation by Mixotrophs, Photoheterotrophs, and Heterotrophs

Because they fashion some monomeric building blocks by catabolism and acquire others preformed from the external environment, heterotrophs use much of the ATP that they generate catabolically for polymerization reactions as in the formation of proteins, polysaccharides, nucleotides and nucleic acids, lipids, and others. Mixotrophs and photoheterotrophs perform anabolic reactions that are similar to those performed by chemolithotrophs, photolithotrophs, and/or heterotrophs.

So far, nothing is known of biochemical steps used by algae or protozoa in forming inorganic polymers such as polysilicate. Such a polymerization is expected to involve the consumption of ATP or its equivalent.

6.6 MICROBIAL MINERALIZATION OF ORGANIC MATTER

Microbes play a major role in the transformation of organic matter in the upper lithosphere (soils, sediments, deep subsurface) and in the hydrosphere (oceans and bodies of fresh water). Because biological availability of carbon as well as of other nutritionally vital inorganic elements in the biosphere is limited, it is essential that these elements be recycled for the continuation of life. This recycling requires complete degradation of dead organic matter into inorganic matter, whether the dead organic matter is in the form of remains of dead organisms or metabolic wastes. This process is called **mineralization**. It may be aerobic or anaerobic.

In *aerobic mineralization*, organic matter is completely degraded (oxidized) to CO_2 and H_2O and if N, S, and P are present in the original organic molecule, to NO_3^-, SO_4^{2-}, PO_4^{3-}, and so on. The process by which this mineralization comes about is aerobic respiration, i.e., oxygen serves as terminal electron acceptor in the oxidations. In many instances of aerobic mineralization, a single microorganism may be responsible for the complete degradation of a compound. In other instances, however, consortia of two or more organisms may collaborate in the process, especially in the degradation of polymers because only one or a few consortium members produce the enzymes needed for depolymerization, which occurs extracellularly in general.

In *anaerobic mineralization*, products of complete degradation of organic matter are CH_4 and/or CO_2, H_2, NH_3, H_2S, PO_4^{3-}, and so on. The process by which this degradation is accomplished may be anaerobic respiration in which NO_3^-, Fe(III), Mn(IV), SO_4^{2-}, and CO_2 among other reducible entities serve as

terminal electron acceptors instead of O_2, and may involve a single microorganism or a succession of different ones. In a succession of microorganisms, one group may provide extracellular hydrolytic enzymes to depolymerize proteins, carbohydrates, nucleic acids, lipids, and so forth. In the presence of sufficient NO_3^-, Mn(IV), Fe(III), or SO_4^{2-}, denitrifying and manganese-, iron-, or sulfate-reducing bacteria, respectively, may then oxidize the products of hydrolysis to CO_2, H_2O, and so on. In the absence of sufficient NO_3^-, Mn(IV), Fe(III), or SO_4^{2-}, fermenters may convert the products of polymer hydrolysis to organic acids, alcohols, and some CO_2 and H_2. Methanogens may then convert the acetate and the CO_2 plus H_2 to CH_4 (see also Chap. 21). Thus, depending on the availability of suitable terminal acceptors to carry on anaerobic respiration, methanogenesis may be an important process of organic carbon mineralization in soils, sediments, and the deep subsurface.

In some environments, mineralization of organic matter may lag or be absent, with the result that organic matter accumulates. This may be because the organic matter that accumulates is difficult for many microbes to degrade—for instance, lignin in wood. In other cases when the organic matter is unsuitable for fermentation, the accumulation may be the result of limited availability of O_2, NO_3^-, Mn(III) or (IV), Fe(III), SO_4^{2-} or CO_2 as terminal electron acceptors. In still other instances, incomplete degradation of organic matter may be the result of enzymatic deficiencies in the organisms in the environment in which the organic matter is accumulating. In soil, incomplete oxidation of some organic matter results in the formation of an important soil constituent, *soil humus*, a mixture of polymeric substances derived from the partial decomposition of plant, animal, and microbial remains and from microbial syntheses. It is usually recognizable as a brownish-black organic complex, only portions of which are soluble in water; a larger fraction is soluble in alkali. Soil humus includes aromatic molecules, often in polymerized form, whose origin is mostly lignin, bound and free amino acids, uronic acid polymers, free and polymerized purines and pyrimidines, and other forms of bound phosphorus.

Organic matter accumulating in marine sediments has been called *marine humus* because of a similarity in its C/N ratio to that of soil humus (Waksman, 1933) and because of its relative resistance to aerobic microbial decomposition (Waksman and Hotchkiss, 1937; Anderson, 1940). However, more detailed chemical analysis of marine humus indicates differences from soil humus, which is not surprising in view of differences in origin (Jackson, 1975; Moore, 1969, p. 271). Whereas soil humus is formed mainly from plant remains, typical marine humus in sediments far from land derives mainly from phytoplankton remains and fecal residues. Marine humus from three northern Pacific sediments contains 0.14–0.34% organic matter, including 20–1145 ppm alkali-soluble humic acids, 40–55% of benzene-soluble bitumen, and 50–180 ppm of amino acids. The remainder is kerogen, a material insoluble in aqueous and nonpolar solvents (Palacas et al., 1966). The organic matter of deep-sea sediments contains

a fraction that, although refractory to microbial attack in situ, is readily attacked by microbes when brought to the surface (see, e.g., Ehrlich et al., 1972). Presumably, high hydrostatic pressure (>300 atm or 303.9 bar) and low temperature (< 4°C) prevent rapid microbial in situ decomposition (Jannasch and Wirsen, 1973; Wirsen and Jannasch, 1975). Metabolizable organic matter in shallow-water sediments will undergo more complete decomposition provided that it does not accumulate too rapidly.

6.7 MICROBIAL PRODUCTS OF METABOLISM THAT CAN CAUSE GEOMICROBIAL TRANSFORMATIONS

Many heterotrophic bacteria, whether aerobic, facultative, or anaerobic, form significant quantities of organic acids among the products from their catabolism in addition to CO_2. At least some of the CO_2 will react with water to form carbonic acid (H_2CO_3) in aqueous solution. Some chemolithotrophs and photolithotrophs form significant amounts of sulfuric or nitric acids, depending on the substrate they use as their source of energy and/or reducing power. These acids may react chemically with certain minerals resulting in their partial or complete dissolution or alteration (diagenesis) (see Chaps. 8 and 9). Other heterotrophs, when growing at the expense of nitrogenous carbon and energy sources such as protein or peptides, generate ammonia, which forms NH_4OH, a base, in aqueous solution. This base can solubilize some silicates.

Various prokaryotes and some eukaryotes form ligands that can complex inorganic ions. Some, like siderophores, are very specific as to the ion they complex. In the case of minerals, when the ion complex formed by the ligand is more stable than the source of the ion, the ligand can withdraw the ion from the mineral resulting in its diagenesis or dissolution (see Chaps. 9, 11, and 15).

Some bacteria can form strong reductants, for example, Fe^{2+} by reducing FeOOH or H_2S by reducing SO_4^{2-}. If the reductant formed is Fe^{2+}, it may then react nonbiologically with a reducible mineral such as pyrolusite (MnO_2), dissolving it by reducing the Mn(IV) to Mn^{2+} (see Chap. 16). This reaction is favored by acid pH. If the reductant formed is H_2S, it may react with FeOOH by reducing it to FeS (see Chap. 15). Acidophilic iron oxidizers such as *Thiobacillus ferrooxidans* produce Fe^{3+} from Fe^{2+}. The Fe^{3+} may chemically oxidize a metal sulfide like CuS, dissolving it by forming $CuSO_4$ (see Chap. 19).

6.8 PHYSICAL PARAMETERS THAT INFLUENCE GEOMICROBIAL ACTIVITY

Temperature is an important parameter that influences geomicrobial activity. In fact, it influences biological activity in general. This is because biochemical

reaction rates, like all chemical reaction rates, increase with a rise in temperature, except that with enzyme-catalyzed reactions this positive response is confined to a relatively narrow temperature range because of the limited heat stability of enzyme proteins. Proteins denature, i.e., they become structurally randomized above a maximum temperature. If they are enzymes, this means that they lose their catalytic activity. Denaturation of some enzymes at temperatures slightly above the temperature maximum can be prevented if accompanied by a moderate increase in hydrostatic pressure (Haight and Morita, 1962).

The lipid phase of cell membranes also responds to temperature. It is more fluid at higher temperature than at lower temperature. A certain degree of membrane fluidity is essential for proper cell functioning, which cells control by adjusting the degree of saturation of the fatty acids in their membrane lipids. The more saturated the fatty acids of a given chain length in membrane lipid, the higher the temperature required for a desirable degree of fluidity, and conversely, the more unsaturated these fatty acids, the lower the temperature required to maintain a similar degree of fluidity.

At present, life is known to exist in a temperature range from slightly below 0°C to as high as +130°C. No organism exists, however, that spans this entire range. That is because proteins and some other structural components of cells require somewhat different compositions and structures for stability and activity for different temperature invervals within the overall temperature range in which life exists. Intact organisms reflect the heat stability range of their enzymes and critical cell structures such as cell membranes by the temperature range in which they grow. Key molecules in organisms with different temperature requirements evidently have different heat labilities (Brock, 1967; Tansey and Brock, 1972). **Psychrophiles** grow in a range from slightly below 0° to about 20°C, with an optimum at 15°C or lower (Morita, 1975). **Psychrotrophs** grow over a wider temperature range than do psychrophiles (e.g., 0–30°C), with an optimum near 25°C. **Mesophiles** are microbes that grow in the range of 10–45°C, with an optimum range for some of about 25–30°C and for others of about 37–40°C. **Thermophiles** are microbes that live in a temperature range of 42–130°C, but the range for any given thermophile is considerably narrower. The temperature optimum for any one thermophilic organism depends on its identity and usually corresponds to the predominant temperature of its normal habitat. *Extreme thermophiles*, those growing optimally above 60°C, seem to be mostly archaea. Generally, thermophilic photosynthetic prokaryotes cannot grow at temperatures higher than 73°C. In contrast, thermophilic eukaryotic algae cannot grow at temperatures higher than 56°C (Brock, 1967, 1974, 1978). Thermophilic fungi generally exhibit temperature maxima around 60°C, and thermophilic protozoa, around 50°C. Only nonphotosynthetic, thermophilic prokaryotes exhibit temperature maxima that may be as high as 130°C. For growth at temperatures at or above the boiling point of water, elevated hydrostatic pressure is needed to keep the water liquid. Liquid water is a requirement for life.

The parameters of pH and E_h also exert important influences on geomicrobial activity, as they do on biological activity in general. Each enzyme has its characteristic pH optimum, and E_h optimum in the case of redox enzymes, at which it catalyzes most efficiently. That is not to say that in the cell or, in the case of extracellular enzymes, outside the cell, an enzyme necessarily operates at its optimal pH and E_h. The interior of living cells tends to have a pH around neutrality and an E_h that is higher than that of its external environment. Enzymes with higher or lower pH optima will operate at less than optimal efficiency. This helps a cell to integrate individual enzyme reactions in a sequence. Changes in external pH within the physiological range of a microorganism does not affect a cell's internal pH because of its plasma membrane barrier and its ability to control internal pH. Extreme changes will, however, affect a cell adversely.

Environmental pH and E_h control the range of distribution of microorganisms [see, however, Ehrlich (1993) for environmental significance of E_h]. Figure 6.13 summarizes the pH and E_h ranges in which certain microbial groups are found to grow. An important feature shown in the diagram is the prevalence of iron-oxidizing bacteria and, to some extent, of thiobacteria in environments of relatively reduced potential and elevated pH.

As mentioned in Chapter 5, hydrostatic pressures in excess of 405 bar (400 atm) at a fixed physiologically permissive temperature below the boiling

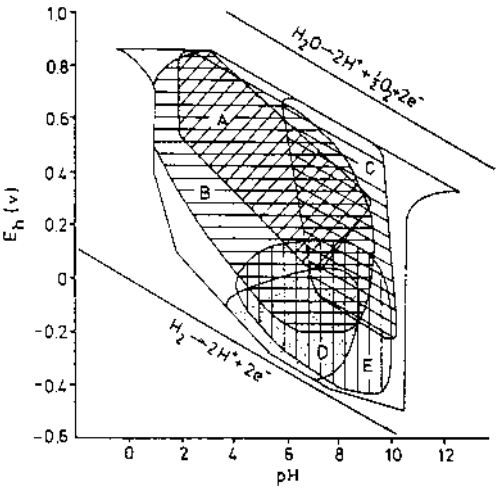


FIG. 6.13 Environmental limits of E_h and pH for some bacteria. A, "iron bacteria"; B, thiobacteria; C, denitrifying bacteria; D, facultative and anaerobic heterotrophic bacteria and methanogens; E, sulfate-reducing bacteria. (Adapted with permission from Baas Becking et al., Geol 68:243–284. Copyright 1960.)

point of water generally prevent growth of *nonbarophilic* microbes. Pressures between 203 and 405 bar (200 and 400 atm) at such a temperature tend to interfere reversibly with cell division of bacteria (ZoBell and Oppenheimer, 1950). Barophilic organisms can grow at pressures above 405 bar (400 atm) at physiologically permissive temperatures. *Facultative barophiles* grow progressively more slowly with increasing pressure, whereas *obligate barophiles* grow best at or near the pressure and temperature of their native environment and grow progressively more slowly with decreasing pressure and usually not at all at atmospheric pressure at the same temperature (Yayanos et al., 1982). The growth-inhibiting effect of hydrostatic pressure is attributable to its effect on protein synthesis (Schwarz and Landau, 1972a, 1972b; Pope et al., 1975; Smith et al., 1975). Many other biochemical reactions are much less pressure-sensitive (Pope and Berger, 1973) (see also Chap. 5).

6.9 SUMMARY

Microbes may make a geologically significant contribution to lithification, mineral formation, mineral diagenesis, and sedimentation, but not to volcanism, tectonic activity, orogeny, or wind and water erosion. They may act as agents of concentration, dispersion, or fractionation of mineral matter. Their influence may be direct, through action of their enzymes, or indirect, through chemical action of their metabolic products, through passive concentration of insoluble substances on their cell surface, and through alteration of pH and E_h conditions in their environment. Their metabolic influence may involve anabolism or catabolism under aerobic or anaerobic conditions. Respiratory activity of prokaryotes may cause oxidation or reduction of certain inorganic compounds, resulting in their precipitation, often as minerals, or in their solubilization. Chemolithotrophic and some mixotrophic bacteria can obtain useful energy from the oxidation of some inorganic substances, such as H_2, Fe(II), Mn(II), H_2S, S^0, and others. Photolithotrophic bacteria can use H_2S as a source of reducing power in the assimilation of CO_2 and, in the process, deposit sulfur. Anaerobically respiring organisms, which use any of various oxidized inorganic substances as terminal electron acceptors, are important in the mineralization of organic matter in environments devoid of atmospheric oxygen. Mineralization of organic matter by microbes under aerobic conditions leads to the formation of CO_2, H_2O, NO_3^-, SO_4^{2-}, PO_4^{3-}, and so on, and under anaerobic conditions it leads to the formation of CH_4, CO_2, NH_3, H_2S, PO_4^{2-}, and so on. Some organic matter is refractory to mineralization under anaerobic conditions and is microbially converted to humus. All microbial activities are greatly influenced by pH and E_h conditions in the environment.

REFERENCES

Anderson DQ. 1940. Distribution of organic matter in marine sediments and its availability to further decomposition. J Mar Res 2:225–235.

Atlas RM. 1997. Principles of Microbiology. Boston, MA: WCB/McGraw-Hill.

Baas Becking LGM, Kaplan IR, Moore D. 1960. Limits of the natural environment in terms of pH and oxidation-reduction potentials. J Geol 68:243–284.

Bak F, Cypionka H. 1987. A novel type of energy metabolism involving fermentation of inorganic sulfur compounds. Nature (Lond) 326:891–892.

Bak F, Pfennig N. 1987. Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. Arch Microbiol 147:184–189.

Beard BL, Johnson CM, Cox L, Sun H, Nealson KH, Aguilar C. 1999. Iron isotope biosignatures. Science 285:1889–1892.

Beh M, Strauss G, Huber R, Stetter K-O, Fuchs G. 1993. Enzymes of the reductive citric acid cycle in the autotrophic eubacterium *Aquifex pyrophilus* and in the archaebacterium *Thermoproteus neutrophilus*. Arch Microbiol 160:306–311.

Beveridge TJ. 1989. Metal ions and bacteria. In: Beveridge TJ, Doyle RJ, eds. Metal Ions and Bacteria. New York: Wiley, pp 1–29.

Beveridge TJ, Doyle RJ, eds. 1989. Metal Ions and Bacteria. New York: Wiley.

Beveridge TJ, Meloche JD, Fyfe WS, Murray RGE. 1983. Diagenesis of metals chemically complexed to bacteria: Laboratory formation of metal phosphates, sulfides and organic condensates in artificial sediments. Appl Environ Microbiol 45:1094–1108.

Blaut M, Gottschalk G. 1984. Proton motive force-driven synthesis of ATP during methane formation from molecular hydrogen and formaldehyde or carbon dioxide in *Methanosarcina barkeri*. FEMS Microbiol Lett 24:103–107.

Brock TD. 1967. Life at high temperatures. Science 158:1012–1019.

Brock TD. 1974. Biology of Microorganisms. 2nd ed. Englewood Cliffs, NJ: Prentice-Hall.

Brock TD. 1978. Thermophilic Microorganisms and Life at High Temperatures. New York: Springer-Verlag.

Brock TD, Madigan MT. 1988. Biology of Microorganisms. 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Butsch BM, Bachofen R. 1984. The membrane potential in whole cells of *Methanobacterium thermoautotrophicum*. Arch Microbiol 138:293–298.

De La Rocha CL, Brzezinski MA, DeNiro MJ. 1997. Fractionation of silicon isotopes by marine diatoms during biogenic silica formation. Geochim Cosmochim Acta 61:5051–5056.

de Vrind-de Jong EW, de Vrind JPM. 1997. Algal deposition of carbonates and silicates. In: Banfield JF, Nealson KH, eds. Geomicrobiology: Interactions Between Microbes and Minerals. Rev Mineral, Vol 35. Washington, DC: Mineral Soc Am, pp 267–307.

Doetsch RN, Cook TM. 1973. Introduction to Bacteria and Their Ecobiology. Baltimore, MD: Univ Park Press.

Doyle RJ. 1989. How cell walls of gram-positive bacteria interact with metal ions. In: Beveridge TJ, Doyle RJ, eds. Metal Ions and Bacteria. New York: Wiley, pp 275–293.

Duplessy J-C, Blanc P-L, Be AWH. 1981. Oxygen-18 enrichment of planktonic foraminifera due to gametogenic calcification below the euphotic zone. Science 213:1247–1250.

Eden G, Fuchs G. 1983. Autotrophic CO_2 fixation in *Acetobacterium woodii*. II. Demonstration of enzymes involved. Arch Microbiol 135:68–73.

Ehrlich HL. 1964. Bacterial oxidation of arsenopyrite and enargite. Econ Geol 59:1306–1312.

Ehrlich HL. 1978. Inorganic energy sources for chemolithotrophic and mixotrophic bacteria. Geomicrobiol J 1:65–83.

Ehrlich HL. 1993. Bacterial mineralization of organic carbon under anaerobic conditions. In: Bollag J-M, Stotzky G, eds. Soil Biochemistry, Vol 8. New York: Marcel Dekker, pp 219–247.

Ehrlich HL. 1996. How microbes influence mineral growth and dissolution. Chem Geol 132:5–9.

Ehrlich HL. 1999. Microbes as geologic agents: Their role in mineral formation. Geomicrobiol J 16:135–153.

Ehrlich HL. 2000. Ocean manganese nodules: Biogenesis and bioleaching possibilities. Miner Metall Process 17:121–128.

Ehrlich HL, Ghiorse WC, Johnson GL II. 1972. Distribution of microbes in manganese nodules from the Atlantic and Pacific Oceans. Dev Ind. Microbiol 13:57–65.

Ehrlich HL. Yang SH, Mainwaring JD Jr. 1973. Bacteriology of manganese nodules. VI. Fate of copper, nickel, cobalt and iron during bacterial and chemical reduction of the manganese(IV). Z Allgem Mikrobiol 13:39–48.

Ehrlich HL, Ingledew WJ, Salerno JC. 1991. Iron- and manganese-oxidizing bacteria. In: Shively JM, Barton LL, eds. Variations in Autotrophic Life. London: Academic Press, pp 147–170.

Eisenreich W, Strauss G, Werz U, Fuchs G, Bacher A. 1993. Retrobiosynthetic analysis of carbon fixation in the phototrophic eubacterium *Chloroflexus auriantiacus*. Eur J Biochem 215:619–632.

Estep MF, Hoering TC. 1980. Biogeochemistry of the stable hydrogen isotopes. Geochim Cosmochim Acta 44:1197–1206.

Evans MCW, Buchanan BB, Arnon DI. 1966. A new ferrodoxin-dependent carbon reduction cycle in a photosynthetic bacterium. Proc Natl Acad Sci USA 55:928–934.

Ferris FG. 1989. Metallic ion interactions with the outer membrane of gram-negative bacteria. In: Beveridge TJ, Doyle RJ, eds. Metal Ions and Bacteria. New York: Wiley, pp 295–323.

Ferris JG. 1993. Methanogenesis. Ecology, Physiology, Biochemistry and Genetics. New York: Chapman and Hall.

Finster K, Liesack W, Thamdrup B. 1998. Elemental sulfur and thiosulfate disproportionation by *Desulfocapsa sulfoexigens* sp. nov., a new anaerobic bacterium isolated from marine surface sediment. Appl Environ Microbiol 64:119–125.

Geesey GG, Jang L. 1989. Interactions between metal ions and capsular polymer. In: Beveridge TJ, Doyle RJ, eds. Metal Ions and Bacteria. New York: Wiley, pp 325–357.

Gottschalk G. 1985. Bacterial Metabolism, 2nd ed. New York: Springer-Verlag.

Graham LA. 1987. Biochemistry and electron transport of a manganese-oxidizing bacterium. PhD Dissertation. Rensselaer Polytechnic Inst, Troy, NY.

Haight RD, Morita RY. 1962. Interaction between the parameters of hydrostatic pressure and temperature on aspartase of *Escherichia coli*. J Bacteriol 83:112–120.

Herter S, Farfsing J, Gad'on N, Rieder C, Eisenreich W, Bacher A, Fuchs G. 2001. Autotrophic CO_2 fixation by *Chloroflexus aurianticus*: Study of glyoxalate formation and assimilation via the 3-hydroxypropionate cycle. J Bacteriol 183:4305–4316.

Hinkle PC, McCarty RE. 1978. How cells make ATP. Sci Am 238:104–123.

Ingledew WJ. 1982. *Thiobacillus ferroxidans*. The bioenergetics of an acidophilic chemolithotroph. Biochim Cosmochim Acta 683:89–117.

Ivanovsky RN, Krasilnikova EN, Fal YI. 1993. A pathway of the autotrophic CO_2 fixation in *Chloroflexus aurianticus*. Arch. Microbiol. 159:257–264.

Jackson BE, McInerney MJ. 2000. Thiosulfate disproportionation by *Desulfotomaculum thermobenzoicum*. Appl Environ Microbiol 66:3650–3653.

Jackson TA. 1975. Humic matter in natural waters and sediments. Soil Sci 119: 56–64.

Jannasch HW, Wirsen CO. 1973. Deep-sea microorganisms: In situ response to nutrient enrichment. Science 180:641–643.

Janssen PH, Schuhmann A, Bak F, Liesack W. 1996. Disproportionation of inorganic sulfur compounds by the sulfate-reducing bacterium *Desulfocapsa thiozymogenes* gen nov., spec. nov. Arch Microbiol 166:184–192.

Lowenstamm HA. 1981. Minerals formed by microorganisms. Science 211:1126–1131.

Moore LR. 1969. Geomicrobiology and geomicrobial attack on sediment organic matter. In: Eglinton G, Murphy MTJ, eds. Organic Geochemistry: Methods and Results. New York: Springer-Verlag, pp 264–303.

Morita RY. 1975. Psychrophilic bacteria. Bacteriol Rev 39:144–167.

Mountford DO. 1978. Evidence for ATP synthesis driven by a proton gradient in *Methanobacterium barkeri*. Biochim Biophys Res Commun 85:1346–1351.

Myers CR, Myers JM. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. J Bacteriol 174:3429–3438.

Onishi T, Meinhardt SW, Yagi T, Oshima T. 1987. Comparative studies on the NADH-Q oxido-reductase segment of the bacteria respiratory chain. In: Kim CH, Tedeschi H, Diwan JJ, Salerno, JC, eds. Advances in Membrane Biochemistry and Bioenergetics. New York: Plenum Press, pp 237–248.

Palacas JG, Swanson VE, Moore GW. 1966. Organic geochemistry of three North Pacific deep sea sediment samples. US Geol Surv Prof Pap 550C, pp C102–C107.

Payne WE, Yang X, Trumpower BL. 1987. Biochemical and genetic approaches to elucidating the mechanism of respiration and energy transduction in *Paracoccus denitrificans*. In: Kim CH, Tedeschi H, Diwan JJ, Salerno, JC, eds. Advances in Membrane Biochemistry and Bioenergetics. New York: Plenum Press, pp 273–284.

Pezacka E, Wood HG. 1984. The synthesis of acetyl-CoA by *Clostridium thermoaceticum* from carbon dioxide, hydrogen, coenzyme A and methyltetrahydrofolate. Arch Microbiol 137:63–69.

Pope DH, Berger LR. 1973. Inhibition of metabolism by hydrostatic pressure: What limits microbial growth? Arch Mikrobiol 93:367–370.

Pope DH, Smith WP, Swartz RW, Landau JV. 1975. Role of bacterial ribosomes in barotolerance. J Bacteriol 121:664–669.

Sakaguchi T, Tomita O. 2000. Bioseparation of lithium isotopes by using microorganisms. Resource Environ Biotechnol 3:173–182.

Schwarz JR, Landau 1972a. Hydrostatic pressure effects on *Escherichia coli*: Site of inhibition of protein synthesis. J Bacteriol 109:945–948.

Schwarz JR, Landau JV. 1972b. Inhibition of cell-free protein synthesis by hydrostatic pressure. J Bacteriol 112:1222–1227.

Smith DW, Strohl WR. 1991. Sulfur oxidizing bacteria. In: Shively JM, Barton LL, eds. Variations in Autotrophic Life. London: Academic Press, pp 121–146.

Smith W, Pope D, Landau JV. 1975. Role of bacterial ribosome subunits in barotolerance. J Bacteriol 124:582–584.

Sprott GD, Bird SE, McDonald IJ. 1985. Proton motive force as a function of cell pH at which *Methanobacterium bryantii* is grown. Can J Microbiol 31:1031–1034.

Stanier RY, Ingraham JL, Wheelis ML, Painter PR. 1986. The Microbial World. 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Strauss G, Fuchs G. 1993. Enzymes of a novel autotrophic CO_2 fixation pathway in the phototrophic bacterium *Chloroflexus auriantiacus*, the 3-hydroxypropionate cycle. Eur J Biochem 215:633–643.

Strohl, WR, Geffers I, Larkin JM. 1981. Structure of the sulfur inclusions envelopes from four beggiatoas. Curr Microbiol 6:75–79.

Stryer L. 1995. Biochemistry. 4th ed. New York: WH Freeman.

Tansey MR, Brock TD. 1972. The upper temperature limit for eukaryotic organisms. Proc Natl Acad Sci USA 69:2426–2428.

Wada E, Hattori A. 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogen compounds by marine diatoms. Geomicrobiol J 1:85–101.

Waksman SA. 1933. On the distribution of organic matter in the sea and chemical nature and origin of marine humus. Soil Sci 36:125–147.

Waksman SA, Hotchkiss M. 1937. On the oxidation of organic matter in marine sediments by bacteria. J Mar Sci 36:101–118.

Wirsen CO, Jannasch HW. 1975. Activity of marine psychrophilic bacteria at elevated hydrostatic pressure and low temperature. Mar Biol 31:201–208.

Woese CR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579.

Wood P. 1988. Chemolithotrophy. In: Anthony C, ed. Bacterial Energy Transduction. London: Academic Press, pp 183–230.

Xavier KB, Da Costa MS, Santos H. 2000. Demonstration of a novel glycolytic pathway in the hyperthermophilic archeon *Thermococcus zilligii* by ^{13}C-labeling experiments and nuclear magnetic resonance analysis. J Bacteriol 182:4632–4636.

Yayanos AA, Dietz AS, Van Boxtel R. 1982. Dependence of reproduction rate on pressure as a hallmark of deep-sea bacteria. Appl Environ Microbiol 44:1356–1361.

ZoBell CE, Oppenheimer CH. 1950. Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. J Bacteriol 60:771–781.

7

Methods in Geomicrobiology

7.1 INTRODUCTION

Geomicrobiological phenomena can be studied in the field (in situ) and in the laboratory (in vitro) as isolated reactions in reaction vessels and/or in microcosms. Field study of a given geomicrobial phenomenon should ideally involve identification and enumeration of the geomicrobially active agents and in situ measurements of their average growth rate. It should also involve chemical and physical identification of the substrates, i.e., reactants that are attacked, and the products that are formed in the geomicrobial process. Further, it should involve measurement of the overall rate at which the process occurs and assessment of the impact of different environmental factors on it. In practice, however, it may happen that a suspected geomicrobial process is no longer operating at a given site but took place in the geologic past. In that instance, the role of the microorganisms in the process has to be reconstructed from microscopic observations (e.g., searching for microfossils associated with the starting compounds, if still present, especially the products of the process). It may also be reconstructed from geochemical observations, such as searching for biomarker ("fingerprint") compounds in sedimentary rock samples that indicate the past existence of an organism or group of organisms that could have been the geochemical agents responsible. If applicable, evidence of isotopic fractionation of a key element relevant to the geomicrobial process should be sought.

In situ observation of an ongoing geomicrobial process should include a study of the setting. In a terrestrial environment, the nature of rocks, soil, or sediment, whichever are involved, and their constituent minerals ought to be considered together with prevailing temperature, pH, oxidation–reduction potential (E_h), sunlight intensity, seasonal cycles, and the source and availability of moisture, oxygen, or other terminal electron acceptors and nutrients. In an aqueous environment, water depth, availability of oxygen or other terminal electron acceptors, turbidity, light penetration, thermal stratification, pH, E_h, chemical composition of the water, nature of the sediment if part of the habitat, and nutrient source and availability should be examined.

In the laboratory, a geomicrobial process can be studied in a **microcosm**. For this, a large sample of the soil, sediment, or rock on or in which the process is occurring is collected. It is placed in a suitable vessel, which may be a flow-through chamber, a glass or plastic column, a battery jar, or another kind of suitable vessel. Filter-sterilized water from the site at which the sample was collected or a synthetic nutrient solution of a composition that approximates qualitatively and quantitatively the nutrient supply available at the sampling site is added intermittently or continuously. The added nutrient solution should displace an equivalent volume of spent solution from the culture vessel. The setup may be placed in the same environment from which the sample was taken, or it may be incubated at the temperature with the illumination and access to air and humidity to which the sample was exposed at the sampling site. Measurement of the concentration of nutrients and products in the influent and effluent critical to the process under study will give a measure of the process rate. Solid products that are not recoverable in the effluent can be identified and measured in representative samples taken from the microcosm. Continuous or intermittent measurement of temperature, pH, E_h, and oxygen availability in the microcosm will give information about any changes in these parameters, some of which may be a result of microbial activity in the microcosm.

The microcosm will probably contain a *mixed population* of bacteria, not all of which are likely to play a role in the geochemical process of interest. Manipulation of the microcosm through qualitative or quantitative change in nutrient supply, adjustment of pH or temperature, or a combination of these factors may cause selective increases of the organisms directly responsible for the geomicrobial process of interest and intensify the process.

In-vitro laboratory study of a geomicrobial process may be done by isolating the responsible microorganism(s) in **pure culture** from a representative sample from the geomicrobially active site. The process originally observed in the field is recreated with the isolate(s) in batch culture and/or continuous culture. Characterization of the process mechanism will involve qualitative and quantitative measurements of the biogeochemical transformation(s). It may include enzymatic study, where appropriate, as well as an assessment of environmental

effects on the in vitro process. In vitro laboratory study may be important in lending support to field interpretations of geomicrobial processes that are occurring at present or have occurred in the past.

7.2 DETECTION AND ISOLATION OF GEOMICROBIALLY ACTIVE ORGANISMS

A geomicrobial process may be the result of a single microbial species or an association of two or more. An association of microbial species is often called a **consortium**. The basis for the association may be **synergism**, in which no one organism is capable of carrying out the complete process but in which each member of the consortium carries out part of the process in a sequential interaction. It is also possible that not all members of an association of microbes contribute directly to an overall geomicrobial process but instead carry out reactions that create environmental conditions relating, for instance, to pH or E_h that facilitate the geomicrobial process under consideration.

Even if a geomicrobial process is the result of a single organism, that organism rarely occurs as a pure culture in the field. It will usually be accompanied by other organisms, which may play no role in the geomicrobial process under study although they may compete with the geomicrobially active agent for living space and nutrients and may even produce metabolites that stimulate or inhibit the geomicrobial agent to a degree. Three types of microorganisms may be found associated with a geomicrobial sample taken in the field: (1) **indigenous organisms**, whose normal habitat is being examined and which include the geomicrobially active organism(s); (2) **adventitious organisms**, which were introduced by chance into the habitat by natural circumstances and which may or may not grow in the new environment but do survive in it; and (3) **contaminants**, which were introduced in manipulating the environment during in situ geomicrobial study or sampling. Distinctions among these groups are frequently difficult to make experimentally.

A criterion for identifying indigenous organisms may be their frequency of occurrence in a given habitat and in similar habitats at different sites. A criterion for identifying adventitious organisms may be their inability to grow successfully in the habitat under study and their lower frequency of occurrence than in their normal habitat. Neither of these criteria is absolute, however. Identification of a contaminant may simply be based on knowledge about the organism concerned that would make its natural existence in the habitat under study unlikely.

In Situ Observation of Geomicrobial Agents

To detect geomicrobially active microorganisms in situ, visual approaches including direct observation with the naked eye, light microscopy, or transmission

or scanning electron microscopy are possible. Direct visual observation is possible only in rare instances, namely when the microbes occur so massively as to be easily seen as, for instance, algal and bacterial mats in hot springs (e.g., in Yellowstone Park; see Brock, 1978) or lichen growth on rocks. In most instances, observations of microbes in their natural habitat require magnification. In soil or sediment, such observations may be made by the buried slide method or the capillary technique of Perfil'ev (Perfil'ev and Gabe, 1969). In the buried slide method a clean microscope slide is inserted in soil or sediment and left undisturbed for a number of days. It is then withdrawn, washed, and suitably stained. A choice of stains includes those that discriminate between living and dead cells and those that do not. Examination with a light microscope, equipped for fluorescence microscopy if fluorescent stains were used (Lawrence et al., 1997), will then reveal microorganisms, especially bacteria and fungi, that became attached to the glass surface during burial (Fig. 7.1).

In the capillary technique one or more glass capillaries with optically flat sides are inserted into soil (pedoscope) or sediment (peoloscope). Each capillary takes up soil or sediment solution and very small soil or sediment particles that become the culture medium for microbes that entered the capillary lumen either at the moment of emplacement and/or subsequently. The capillaries can be periodically withdrawn and their content examined under a light microscope. The capillaries may also be perfused with special nutrient solutions. The capillaries

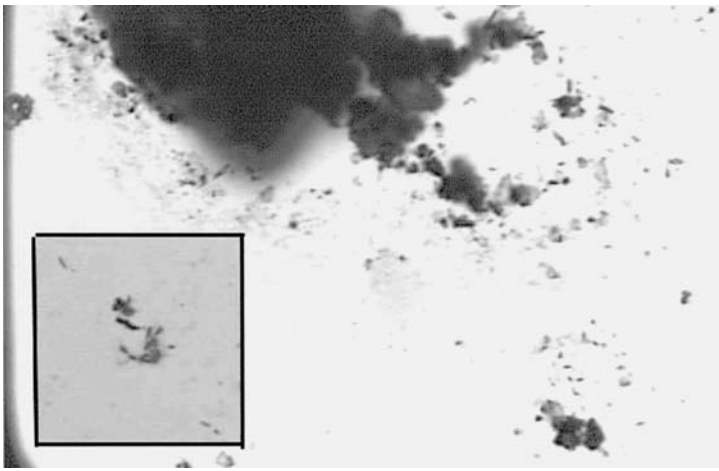


FIG. 7.1 Demonstration of microbes in soil by the buried slide method. Slide was buried for one week. After withdrawal from soil and gentle washing, it was stained with crystal violet. Main view is of isolated shorter and longer rods, and some cocci near soil particles. Inset shows a clump of rods in slime (biofilm?) (×2200).

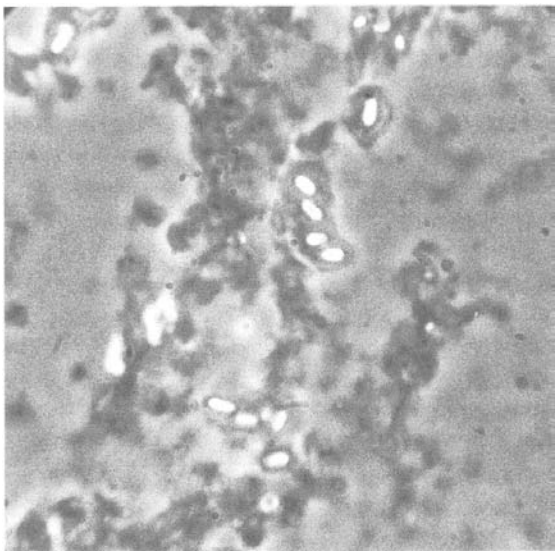


FIG. 7.2 Microbial development in a capillary tube inserted into lake sediment contained in a beaker and incubated at ambient temperature (×5720). The oval, refractile structures are bacterial spores.

permit observation of trapped microbes in a living or nonliving state (Fig. 7.2). Using this technique, Perfil'ev and Gabe (1965) discovered several previously unknown bacteria in soil and sediment, including *Metallogenium*, *Kuznetsova*, and *Caulococcus*.

Although the buried slide and capillary methods give an indication of some of the organisms present in the soil or sediment where the slides or capillaries were inserted, they do not indicate whether the organisms that are seen resided preferentially on the soil or sediment particles or in the pore fluid. To make that kind of determination, direct observation of samples of pore fluid and of soil or sediment particles is necessary. To observe organisms in pore fluids, axenically collected samples of fluid can be filtered and any microbial cells in the fluid deposited on suitable filter membranes, which are then stained and subsequently examined microscopically (Clesceri et al., 1989).

To observe microbes directly on soil or sediment particles or on rock fragments, fluorescence microscopy may be used in conjunction with staining with fluorescent dyes or with fluorescently labeled antibodies if specific microbes are being sought (Fig. 7.3) (Bohlool and Brock, 1974; Casida, 1962, 1965; 1971; Edwards et al., 1999; Eren and Pramer, 1966; Huber et al., 1985; Kepner and

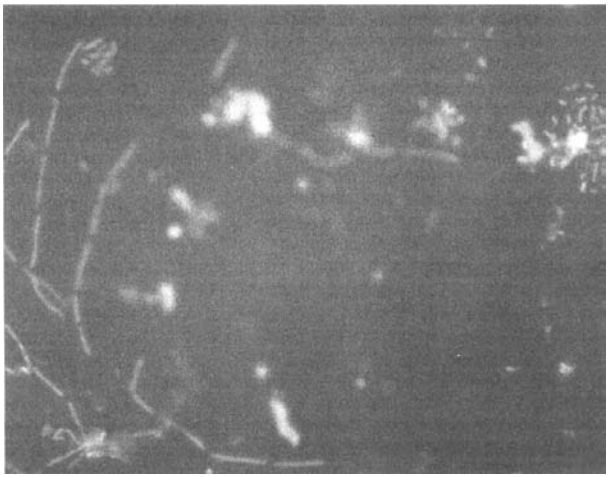


FIG. 7.3 Bacteria growing on the surface of 304L stainless steel immersed in tap water (Troy, NY) for 3 days, stained with fluorescein isothicyanate. (Courtesy of Daniel H. Pope.)

Pratt, 1994; Muyzer et al., 1987; Schmidt and Bankole, 1965). Another approach is to examine sectioned samples by transmission electron microscopy, which is also very useful in identifying fossilized microbes (Barker and Banfield, 1998; Ghiorse and Balkwill, 1983; Jannasch and Mottl, 1985; Jannasch and Wirsen, 1981; Schopf, 1983). Still another approach is the use of scanning electron microscopy (see, e.g., Fig 15.15) (Jannasch and Wirsen, 1981; Sieburth, 1975; LaRock and Ehrlich, 1975; Edwards et al., 1999).

Recent advances in the techniques of molecular biology have led to the development of powerful methods for identifying microorganisms and studying their phylogeny. These methods have been adapted to locate and enumerate microorganisms in environmental samples, even microorganisms that, though viable, have not been cultured in the laboratory (Ward et al., 1990; Stahl, 1997).

Approaches in identifying any pure cultures of bacteria include DNA/DNA hybridization, DNA/RNA hybridization, DNA fingerprinting, and 16S ribosomal RNA (16S rRNA) analysis (Saylor and Layton, 1990; Amann et al., 1990; Stahl, 1997). DNA fingerprinting involves extracting of DNA from a pure culture of the unknown organism, digesting the DNA with specific restriction enzymes, which cleave it into polynucleotide fragments, subjecting the digest to electrophoresis, and comparing the resultant pattern of different nucleotide fragments with patterns obtained in the same way from DNA extracted from pure cultures of known organisms.

The recognition that a 16S rRNA molecule of prokaryotes possesses both highly conserved nucleotide sequences and sequences that are variable to different extents has provided a handle for distinguishing between the domains of the Bacteria and the Archaea and between groups of organisms within each of these two domains. Thus, based on the levels of variability, distinctions can be made at the phylogenetic, generic, and species levels (e.g., Ward et al., 1992). This molecular information makes possible the construction of phylogenetic trees that indicate relatedness of different organisms at the group, generic, or species level. Because any 16S rRNA sequence of a new prokaryote once established is entered into a databank (e.g., Ribosomal Database Project or RDP; GenBank), it is possible to compare 16S rRNA sequences from a new isolate with those in the database to determine if it has been previously reported and to identify its closest relatives. Probes based on specific 16S rRNA nucleotide sequences can be applied directly to intact cells that have been treated to make them permeable to the probes and allow hybridization between the probes and the corresponding nucleotide sequence in the ribosomal 16S rRNA. When such probes are labeled with a fluorescent dye or with a radioactive isotope, cells that have reacted with the probe can be readily located by fluorescence microscopy or autoradiography, respectively (Amann et al., 1990, 1992, 1995; Braun-Howland et al., 1992, 1993; DeLong et al., 1989; Giovannoni et al., 1988; Jurtshuk et al., 1992; Tsien et al., 1990; Ward et al., 1990). This technique is called FISH (fluorescence in situ hybridization). The 16S rRNA probes can be group-, genus- and/or species-specific.

Advances in molecular biological techniques in the last decade have enabled the detection of organisms in microbial communities (mixed cultures) without isolating each member in pure culture. By the use of the polymerase chain reaction (PCR) technique with primers targeting 16S rRNA genes, it is possible to amplify 16S rRNA genes in the DNA extracted from the community. The resultant mixture of 16S rDNAs must then be resolved. One approach employs cloning. The 16S rDNA in each clone is reamplified by PCR and the nucleotide sequence of the rRNA is determined. Another approach to resolving the 16S rDNA mixture derived from amplification of the DNA extract is to use denaturing gradient gel electrophoresis (DGGE), which can separate fragments of the same length that differ by only one or two nucleotides. Attempts can then be made to hybridize rDNA bands in the electrophoretic patterns with oligonucleotide probes from known organisms and thus identify the organism corresponding to the band that successfully hybridized with a probe. The bands from the electrophoresis gels can also be eluted, purified if needed, re-amplified, and their nucleotide sequences determined (Ward et al., 1992, 1998; Muyzer et al., 1993; Stahl, 1997; Madigan et al., 2000). Such sequences can then be compared to sequences in an appropriate database to determine if they were previously reported. Applying either of these techniques to environmental samples has led

to the discovery that most of the microbial flora in samples from a great variety of sources has never so far been isolated, cultivated, and characterized in the laboratory.

Molecular techniques for identifying bacteria have their caveats. For a discussion, see, for instance, Stahl (1997) and references therein.

Sampling

To determine the nature of a geomicrobially active organism in terms of its morphology, physiology, and particular geomicrobial contribution, it should be isolated and cultivated in the laboratory if possible. Some bacterial cultures have had to be studied in the laboratory in the form of enrichments because no method had been found to cultivate them in pure culture. Samples, whatever their nature, brought to the laboratory must be obtained under conditions as aseptic as possible, i.e., with very little or preferably no contamination. Working surfaces of sampling tools should be thoroughly washed and alcohol-flamed. If a rock outcrop is to be sampled, use of a rock hammer or chisel may be required. If the interior of a small rock specimen is to be sampled in the laboratory, the rock surface should be sterilized if possible. This can be accomplished by using disinfectant such as carbol-gentian violet-methanol spray (Bien and Schwartz, 1965) or by flaming briefly with a propane torch or, if done in the laboratory, for 1 min with a bunsen flame (Weirich and Schweisfurth, 1985). If a sampling device cannot be sterilized, the sample it gathers should be subsampled to obtain an aliquot that is least likely to have been contaminated. Rock chips should be collected in sterile plastic containers. If the collection is done manually, the hands should be covered by sterile surgical gloves. Weirich and Schweisfurth (1985) devised a special method for obtaining an undisturbed core of rock with a hollow drill under sterile conditions and with cooling by sterile tap water. The extracted rock core was aseptically cut into sections with a flamed chisel, and each section was then aseptically crushed in a flamed mortar mill in sterile dispersing solution and microbiologically tested.

To sample the terrestrial subsurface down to 3000–4000 m or more, drilling methods have been devised that depend on the use of special drilling equipment that causes minimal if any contamination in the collection of samples (cores) (Phelps and Russell, 1990; Pederson, 1993; Griffin et al., 1997). One method uses modified wireline coring tools, with cores collected in Lexan- or PVC-lined barrels. The drill rig, rods, and tools are steam-cleaned. The drilling fluid system includes a recirculation tank, the drilling fluid being chlorinated water. Tracers such as potassium bromide, the dye rhodamine T, fluorescent beads ($\sim$2 μm diameter), and perfluorocarbons added to the drilling fluid aid in determining to what extent, if any, cores were contaminated in the drilling. The assessment is made by measuring the extent of their presence, if any, in the cores. The extent of

bacterial contamination can be determined by quantitative enumeration of bacteria such as coliforms that were not expected as part of the normal flora of the core, the enumeration being done on the drilling fluid and core samples (Beeman and Suflita, 1989). If anaerobes as well as aerobes are sought in subsurface samples, the cores should be kept from access to air and be processed in an oxygen-free atmosphere. Subsamples may than be tested for aerobes and anaerobes by appropriate culture techniques.

Soil and sediment samples from shallow depths on land surfaces may be collected manually with an auger or other coring device, observing aseptic conditions. Cores should be subdivided aseptically for sampling at different depths. If the cores cannot be obtained with a sterilized sampling device, they should be subsampled so as to obtain the least contaminated samples.

To obtain aquatic samples, special gear may be required. Water samples at any given depth below the surface, including deep-water samples, may be obtained with a Van Dorn sampler. It consists of a piece of large-diameter plastic tube open at both ends and mounted on a cable or rope in such a way that it can be lowered vertically in a water column. While the device is being lowered, water will pass through it. When a desired depth has been reached, the plastic tube is closed at both ends with rubber closures, which are activated by a spring mechanism that can be released by a messenger (brass weight) that is allowed to slide down on the cable or rope to which the sampler is attached. Below-surface water samples can also be collected with a Niskin sampler, which consists of a collapsible, sterile plastic bag with a tube-like opening mounted between two hinged metal plates. The sampler, with the bag in a collapsed state between the metal plates, is lowered on a cable of rope to a desired depth. A spring mechanism of the sampler is then activated by a messenger that causes the metal plates to open, expanding the bag, which now draws water into it.

Aquatic sediment samples may be obtained with dredging or coring devices. Lake sediment can be collected with an Ekman dredge (Fig. 7.4) or a Peterson dredge (Clesceri et al., 1989, p.10-100) if surface sediment is desired. A corer needs to be used if different levels of a sediment column are to be examined. Ocean surface sediment may be collected by dragging a bucket dredge over a desired area of the ocean floor. Such a sample will, however, consist of combined, mixed surface sediment encompassing the total surface area sampled. To obtain samples representing different sediment depths at a given location, a gravity corer (Fig. 7.5) or a box corer (Fig. 7.6) has to be used. Such devices are rammed into the sediment. Box corers of sufficient cross section provide the least disturbed cores. All cores need to be subsampled to obtain representative, minimally contaminated samples representative of the sediment at a given depth. Large rock fragments or concretions on the sediment surface may be collected with a chain dredge or similar device dragged over the sea bottom in a desired area (Fig. 7.7).

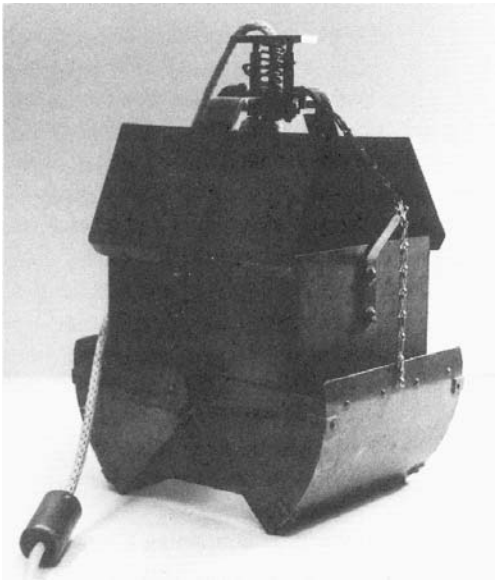


FIG. 7.4 Ekman dredge. The brass messenger on the rope is 5 cm long.

If samples cannot be examined immediately after collection, they should be stored so as to minimize microbial multiplication or death. Cooling a sample is usually the best way to preserve it temporarily in its native state, but the degree of cooling may be critical. Freezing may be destructive to at least some of the microbes. On the other hand, icing may not prevent the growth of psychrophiles or psychrotrophs. The duration of storage before examination should be no longer than absolutely necessary.

Isolation and Characterization of Active Agents in Samples

To study the agents active in a geomicrobial process of interest, culture enrichment and pure culture isolations may be necessary. Not all cultures obtained by these procedures may be geomicrobially active. Each isolate must be tested for its ability to perform the particular geomicrobial activity under investigation. Because some geomicrobial processes are the result of the activity of a microbial consortium, no one of the pure culture isolates may exhibit the desired activity but may have to be tested with others of the isolates in different combinations. Examples of geomicrobial cooperation between microorganisms in microbial manganese oxidation that have been described include (1) the

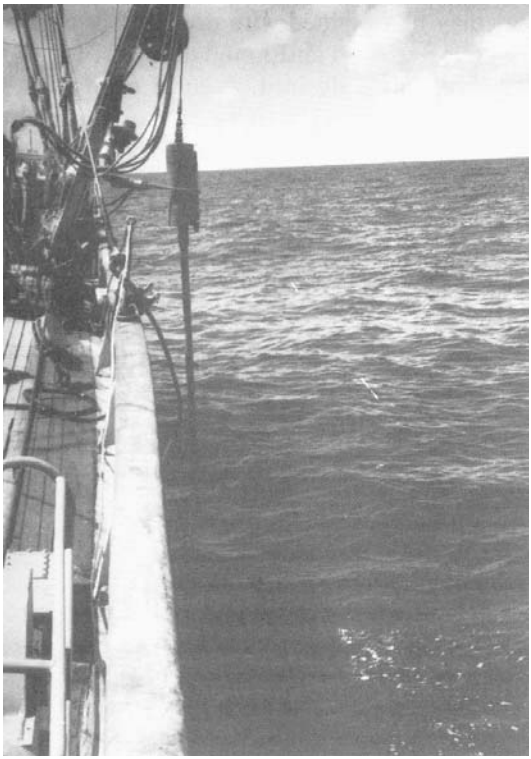


FIG. 7.5 Gravity corer. This is simply a hollow pipe containing a removable plastic liner and having a cutting edge at the lower end with a "core catcher" to retain the sediment core when the device is pulled out of the sediment. A heavy lead weight at the top helps to ram the corer into the sediment when allowed to freefall just above the sediment surface.

bacterium *Metallogenium symbioticum* in association with the fungus *Coniothyrium carpaticum* (Zavarzin, 1961; Dubinina, 1970; but see also Schweisfurth, 1969), (2) the bacteria *Corynebacterium* sp. and *Chromobacterium* sp. (Bromfield and Skerman, 1950; Bromfield, 1956), and (3) two strains of *Pseudomonas* (Zavarzin, 1962).

Enrichment of and isolation from a mixed culture require selective conditions. If a microbial agent with a specific geomicrobial attribute is sought, the selective culture medium should have ingredients incorporated that favor the geomicrobial activity of interest. Apart from special nutrients, special pH, E_h, and temperature conditions may also have to be chosen to favor selective growth of the geomicrobial agents.

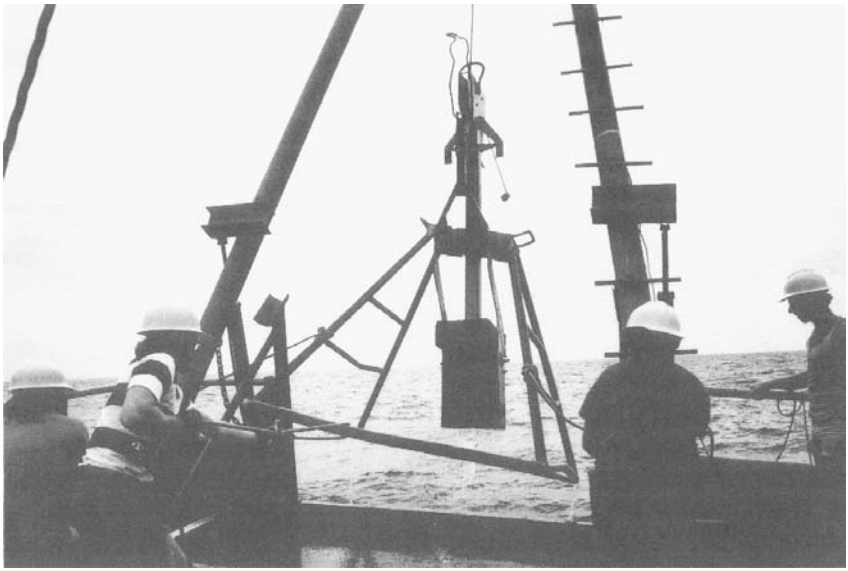
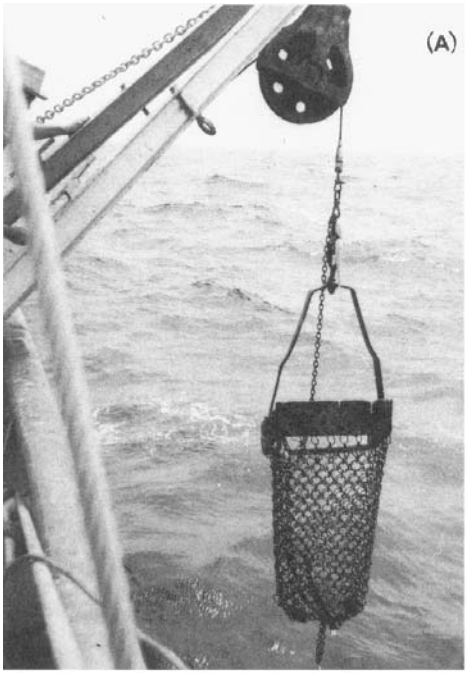


FIG. 7.6 Box corer. After the frame hits bottom, the coring device is forced into the sediment mechanically. (Courtesy of Mark Sand.)

Isolation and characterization of pure cultures from enrichments should follow standard bacteriological technique and will not be discussed further (see, for instance, Gerhardt et al., 1981, 1993; Hurst et al., 1997; and Skerman, 1967 for details).

7.3 IN SITU STUDY OF PAST GEOMICROBIAL ACTIVITY

Geomicrobial activity that occurred in the geologic past can under certain circumstances be identified in terms of isotopic fractionation. Certain prokaryotic and eukaryotic microbes have been shown to distinguish between stable isotopes of elements such as C, H, O, N, S, Si, Li, and Fe. These microbes prefer to metabolize substrates containing the lighter isotopes of these elements (^{12}C in preference to ^{13}C, H in preference to D, ^{16}O in preference to ^{18}O, ^{14}N in preference to ^{15}N, ^{32}S in preference to ^{34}S, ^{28}Si in preference to ^{30}Si, ^{6}Li in preference to ^{7}Li, ^{54}Fe in preference to ^{56}Fe), especially under conditions of slow growth (see Jones and Starkey, 1957; Emiliani et al., 1978; Mortimer and Coleman, 1997; Mandernack et al., 1999; Wellman et al., 1968; Estep and Hoering, 1980; De La Rocha et al., 2000; Sakaguchi and Tomita, 2000; Beard



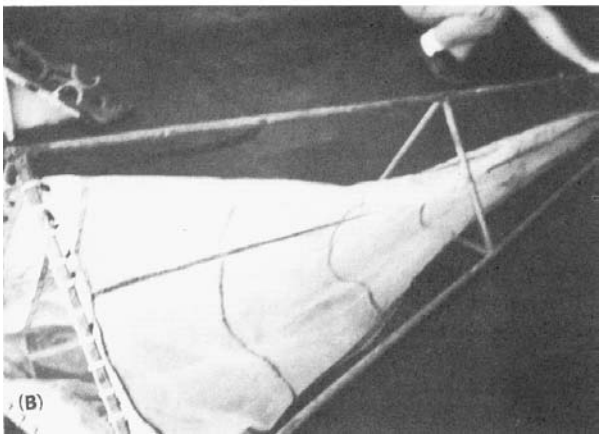


FIG. 7.7 Seafloor samplers. (A) Chain dredge. (B) Dredge for collecting manganese nodules from the ocean floor. A conical bag of nylon netting is attached to a pyramidal frame.

et al., 1999). They distinguish between isotopes of the same element on a kinetic basis in terms of a difference in reaction rate of a specific biochemical step, in terms of a difference in membrane transport rate, or both (see Hoefs, 1997). Thus, products of metabolism will be enriched in the lighter isotope compared to the starting compound or to some reference standard that has not been subjected to isotope fractionation. In practice, isotope fractionation is measured by determining isotopic ratios of the heavier isotope of an element to the lighter, using mass spectrometry and then calculating the amount of isotopic enrichment from the relationship

$$\delta \text{ isotope } (‰) = \frac{\text{isotopic ratio of sample} - \text{isotopic ratio of standard}}{\text{isotopic ratio of standard}} \times 1000 \tag{7.1}$$

If the enrichment value (δ) is negative, the sample tested was enriched in the lighter isotope relative to a reference standard, and if the value is positive, the sample tested was enriched in the heavier isotope relative to a reference standard. Thus, to determine if a certain metal sulfide mineral deposit is of biogenic origin, various parts of the deposit are sampled and ($\delta\ ^{34}S$) values of the sulfide are determined. If the values are generally negative (although the magnitude of the $\delta\ ^{34}S$ may vary from sample to sample and fall in the range of -5 to $-50‰$), the deposit can be viewed as of biogenic origin because a chemical explanation for such ^{32}S enrichment under natural conditions is not likely. If the $\delta\ ^{34}S$ values are positive and fall in a narrow range, the deposit is viewed as being of abiogenic origin.

7.4 IN SITU STUDY OF ONGOING GEOMICROBIAL ACTIVITY

Ongoing geomicrobial activity may be measurable in situ. Such activity may be followed by the use of radioisotopes. For instance, bacterial sulfate reducing activity may be determined by adding a small measured quantity of $Na^{35}SO_4$ to a water, soil, or sediment sample of known sulfate content in a closed vessel. After incubation under in situ conditions, the sample is analyzed for loss of $^{35}SO_4^{2-}$ and buildup of $^{35}S^{2-}$ by separating these two entities and measuring their quantity in terms of their radioactivity. Incubation of the reaction mixture in the closed vessel may be in the water column at the depth from which the water sample was taken. A direct application of this method is that of Ivanov (1968). It allows estimation of the rate of sulfate reduction in the sample without having any knowledge of the number of physiologically active organisms present in it. A modified method is that of Sand et al. (1975). Their method allows an estimation of sulfate-reducing activity in terms of the number of physiologically active bacteria in the sample as

distinct from an estimation of the sum of physiologically active and inactive bacteria. The assay for the estimation of active bacteria can be set up either to measure the percentage of sulfate reduced in a fixed amount of time that is proportional to the logarithm of active cell concentration or to measure the length of time required to reduce a fixed amount of sulfate to sulfide, which is related to the concentration of physiologically active sulfate-reducing bacteria in the sample. Ivanov's method can be adapted to measure the formation of elemental sulfur and sulfates from sulfide by adding $^{32}S^{2-}$ to a sample and, after incubation in a reaction vessel in situ, separating ^{35}S and $^{35}SO_4^{2-}$ and measuring their quantity in terms of their radioactivity.

Microbial action on manganese (Mn^{2+} fixation by biomass; Mn^{2+} oxidation) in situ is another example of a geomicrobial process that can be followed by the use of a radioisotope, $^{54}Mn^{2+}$ in this case (LaRock, 1969; Emerson et al., 1982; Burdige and Kepkay, 1983). One approach is to measure manganese oxidation in terms of decrease in dissolved $^{54}Mn^{2+}$. It assumes that the oxidized manganese is insoluble. Decreases in dissolved $^{54}Mn^{2+}$ are measured on acidified samples of reaction mixture from which the oxidized manganese has been removed by centrifugation. Acidification of samples prior to centrifugation ensures resolubilization of any adsorbed Mn^{2+}. The difference in radioactivity counts between a zero-time sample and a sample taken at a subsequent time is a measure of the amount of Mn^{2+} oxidized by a combination of biological and chemical processes over this time interval. Manganese oxidation due to biological activity alone can be estimated by subtracting separately measured chemical oxidation from the total Mn^{2+} oxidation for a corresponding time interval. An estimate of the amount of chemical oxidation can be obtained from a reaction mixture in which the biological activity is inhibited by autoclaving the sample, by adding one or more chemical inhibitors to the reaction mixture, or by excluding air (if the enzymatic manganese oxidation is oxygen-dependent). This experimental approach has been used to assess the manganese-oxidizing activity in lake sediment (LaRock, 1969). It makes no assumptions about binding of unoxidized or oxidized manganese to the bacterial cells.

Another approach is to measure manganese oxidation in terms of product formation. Manganese binding by metabolically active bacteria in a water sample may be followed in terms of ^{54}Mn accumulation by the cells. A manganese-oxidizing culture is incubated in a suitable reaction mixture containing added $^{54}Mn^{2+}$. After an appropriate length of incubation, the amount of radiomanganese bound by the cells is determined. For this determination, a measured sample of the bacterial suspension is filtered through a 0.2 μm membrane and washed, and the radioactivity on the filter membrane (assumed to be bound to the cells) is determined in a suitable counter. The results of this experiment are then compared to those of a parallel experiment in which the cells were inhibited by a poison such as formaldehyde or a mixture of sodium azide, penicillin G, and

tetracycline-HCl. The difference in radioactivity between these two experiments represents manganese bound by actively metabolizing cells. It includes manganese that was bound as Mn^{2+}, presumably a very minor amount, and that which was bound due to its oxidation by the cells (Emerson et al., 1982). Manganese binding by bacteria in sediment can be followed by a modified form of this method in a special reaction vessel called a peeper (Burdige and Kepkay, 1983). $^{54}Mn^{2+}$ adsorbed by the cells deposited on the filter membrane is displaced by washing with $CuSO_4$ solution. The radioactivity recovered in the wash is then counted. Residual ^{54}Mn associated with the cells (taken as oxidized manganese) is dissolved by washing with hydroxylamine-HCl solution followed by $CuSO_4$ solution, and the radioactivity in the resultant solution is determined. Hydroxylamine-HCl is a reducing agent.

Biological manganese binding in lake sediments may be studied by a method that requires controls in which biological inhibitors are used to account for abiological manganese binding by sediment constituents in the overall manganese budget (Burdige and Kepkay, 1983).

One advantage in using radioisotopes in quantitative assessment of a specific geomicrobial transformation in nature is that their detection is extremely sensitive so that only minute amounts of radiolabeled substrate, which do not significantly change the naturally occurring concentration of the substrate, need to be added. Another advantage is that in cases where the rate of transformation is very slow even though the natural substrate concentration is high, *spiking* the reaction with radiolabeled substrate allows analysis after a relatively brief reaction time because of the sensitivity of radioisotope detection.

The use of radioisotopes is, however, not essential for quantitative assessment in all instances of biogeochemical transformation in a natural environment. Other analytical methods with sufficient sensitivity may be applicable (see, for instance, Jones et al., 1983, 1984; Hornor, 1984; Kieft and Caldwell, 1984; Tuovila and LaRock, 1987).

7.5 LABORATORY RECONSTRUCTION OF GEOMICROBIAL PROCESSES IN NATURE

It is often important to reconstruct a naturally occurring geomicrobial process in the laboratory in order to investigate the mechanisms whereby the process operates. Laboratory reconstruction can permit optimization of a process through application of more favorable conditions than in nature. Examples are the use of a pure culture or a "purified" consortium to eliminate interference by competing microorganisms and the optimization of substrate availability, temperature, pH, E_h, and oxygen and carbon dioxide supply.

The activity of organisms growing on solid substrates such as soils, sediments, rocks, and ore may be investigated in batch culture, in air-lift columns, in percolation columns, or in a chemostat. A batch culture represents a closed system in which an experiment is started with a finite amount of substrate that is continually depleted during growth of the culture. Cell population and metabolic products build up, and changes in pH and E_h are likely to occur. Conditions within the culture are thus continually changing and become progressively less favorable. Batch experiments may be least representative of a natural process, which usually occurs in an open system with continual or intermittent replenishment of substrate and removal of at least some of the metabolic wastes. A culture in an air-lift column (Fig. 7.8) is a partially open system, in that the microbes grow and carry on their biogeochemical activity on a mineral charge in the column. They are continually fed with recirculated nutrient solution from which nutrients are depleted by the organisms. The recirculation removes metabolic products from the solid substrate charge in the column. *Percolation*

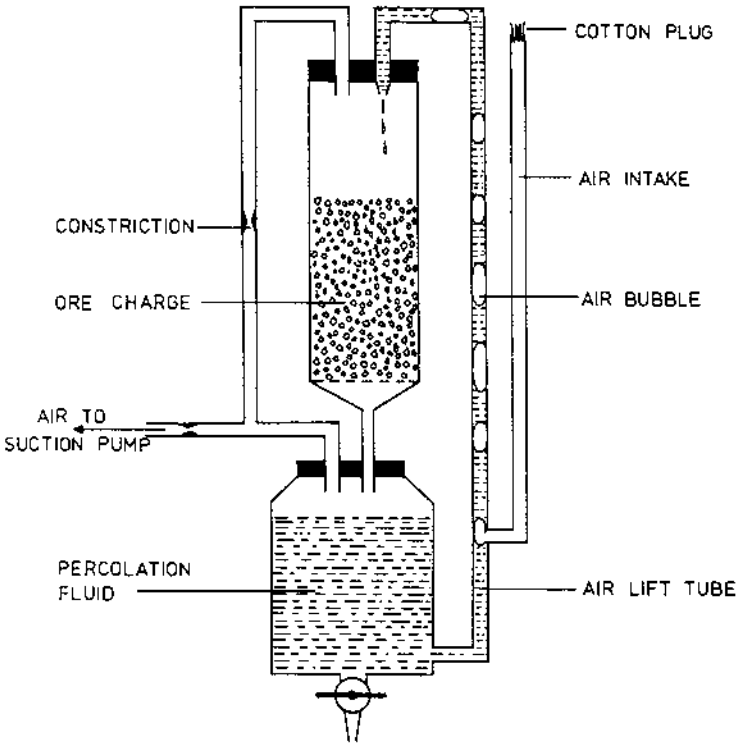


FIG. 7.8 Air-lift column for ore leaching.

columns (Fig. 7.9) are even more open systems than air-lift columns. In them, microbes also grow on the solid substrate charge of the column, but they are fed with nutrient solution that is not recirculated. Thus fresh nutrient solution is added continually or at intervals, and wastes are removed at the same time in the effluent without recirculation, while pH, E_h, and temperature are held constant or nearly so. Steady-state conditions such as exist in a **chemostat** idealize the open culture system. They do not imitate nature, because conditions are too constant. In open systems in nature, some fluctuation in various environmental parameters occurs over time.

The chemostat is a liquid culture system of constant volume. The continuous introduction of fresh nutrient solution at a constant rate keeps the actively growing cell population and the concentration of accumulating metabolic products constant in the culture vessel through medium displacement to maintain constant volume. In other words, steady-state conditions are maintained. This can be expressed mathematically as

$$\frac{dx}{dt} = \mu x - Dx \tag{7.2}$$

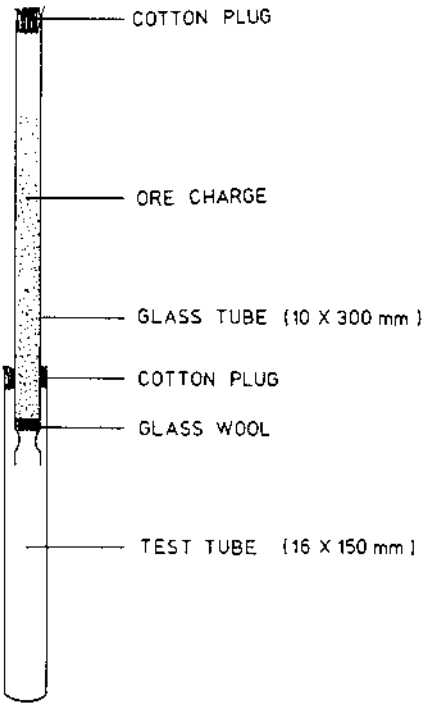


FIG. 7.9 Percolation column for ore leaching.

where dx/dt is the rate of cell population change in the chemostat, μ the instantaneous growth rate constant, D the dilution rate, and x the cell concentration or cell number in the chemostat. The dilution rate is defined as the flow rate (f) of the influent feed or the effluent waste divided by the liquid volume (V) of the culture in the chemostat. Under steady-state conditions, $dx/dt = 0$ and therefore $\mu x = Dx$ (i.e., instaneous growth rate equals dilution rate, $\mu = D$). Under conditions where $D > \mu$, the cell population in the chemostat will decrease over time and may ultimately be washed out. Conversely, if $\mu > D$, the cell population in the chemostat will increase until a new steady state is reached, which is determined by the growth limiting concentration of an essential substrate.

The steady state in the chemostat can also be expressed in terms of the rate change of growth-limiting substrate concentration (ds/dt). This is based on the principle that the rate of change in substrate concentration is dependent on the rate of substrate addition to the chemostat, the rate of washout from the chemostat, and the rate of substrate consumption by the growing organism:

$$\frac{ds}{dt} = D(S_{\text{inflow}} - S_{\text{outflow}}) - \mu(S_{\text{inflow}} - S_{\text{outflow}}) \tag{7.3}$$

where D is the dilution rate, S_{inflow} the substrate concentration entering the chemostat, S_{outflow} the concentration of unconsumed substrate, and μ the instantaneous growth rate constant. At steady state, $ds/dt = 0$. The substrate consumed, i.e., ($S_{\text{inflow}} - S_{\text{outflow}}$,) is related to the cell mass produced (x) according to the relationship

$$x = y(S_{\text{inflow}} - S_{\text{outflow}}) \tag{7.4}$$

in which y is the growth-yield constant (mass of cells produced per mass of substrate consumed). These relationships require modification if a solid substrate is included in the chemostat (see, for instance, Sec. 7.6).

The chemostat can be used, for example, to determine limiting substrate concentrations for growth of bacteria under simulated natural conditions. Thus, the limiting concentrations of lactate, glycerol, and glucose required for growth at different relative growth rates (D/μm) of *Achromobacter aquamarinus* (strain 208) and *Spirillum lunatum* (strain 102) in seawater have been determined by this method (Table 7.1) (Jannasch, 1967). The chemostat principle can also be applied to a study of growth rates of microbes in their natural environment by laboratory simulation (Jannasch, 1969) or directly in their natural habitat. For instance, the algal population in an algal mat of a hot spring in Yellowstone Park, Montana, was found to be relatively constant, implying that the algal growth rate equaled its washout rate from the spring pool. When a portion of the algal mat was darkened by blocking access of sunlight, thereby stopping photosynthesis and thus algal growth and multiplication in that part of the mat, the algal cells were washed out from it at a constant rate after a short lag (Fig. 7.10). The washout rate under these

TABLE 7.1 Threshold Concentrations of Three Growth-Limiting Substrates (mg L^{-1}) in Seawater at Several Relative Growth Rates of Six Strains of Marine Bacteria and the Corresponding Maximum Growth Rates (μ_m) (hr^{-1})

Strain	D/μ_m	Lactate	Glycerol	Glucose
208	0.5	0.5	1.0	0.5
	0.1	0.5	1.0	0.5
	0.005	1.0	5.0	1.0
μ_m:		0.15	0.20	0.34
102	0.3	0.5	No growth	0.5
	0.1	1.0		5.0
	0.05	1.0		10.0
μ_m:		0.45		0.25

Source: Excerpted from Table 2 in Jannasch (1967), with permission.

conditions equaled the growth rate in the illuminated part of the mat. This follows from Eq. (7.2) when $dx/dt = 0$ (Brock and Brock, 1968). Similarly, the population of the sulfur-oxidizing thermophile *Sulfolobus acidocaldarius* has been found to be in steady state in hot springs in Yellowstone Park, implying, as in the case of the algae, that the growth rate of the organism equals its washout rate from the spring. In this instance, the washout rate was measured by following the water turnover rate in terms of dilution rate of a small measured amount of NaCl added to the spring pool (Fig. 7.11). The dilution rate was then translated into the growth rate of *S. acidocaldarius* in the spring (Mosser et al., 1974).

7.6 QUANTITATIVE STUDY OF GROWTH ON SURFACES

The chemostat or its principle of operation is not applicable to all culture situations. In the study of geomicrobial phenomena, the central microbial activity often occurs on the surface of inorganic or organic solids. Indeed, the solid may be the growth-limiting substrate upon which the organism acts. Under these conditions, it may be assumed that the microbial population as it colonizes the surface will increase geometrically once it has settled on it, approximating the relationship

$$\log N = \log N_0 + \frac{\Delta t}{g} \log 2 \tag{7.5}$$

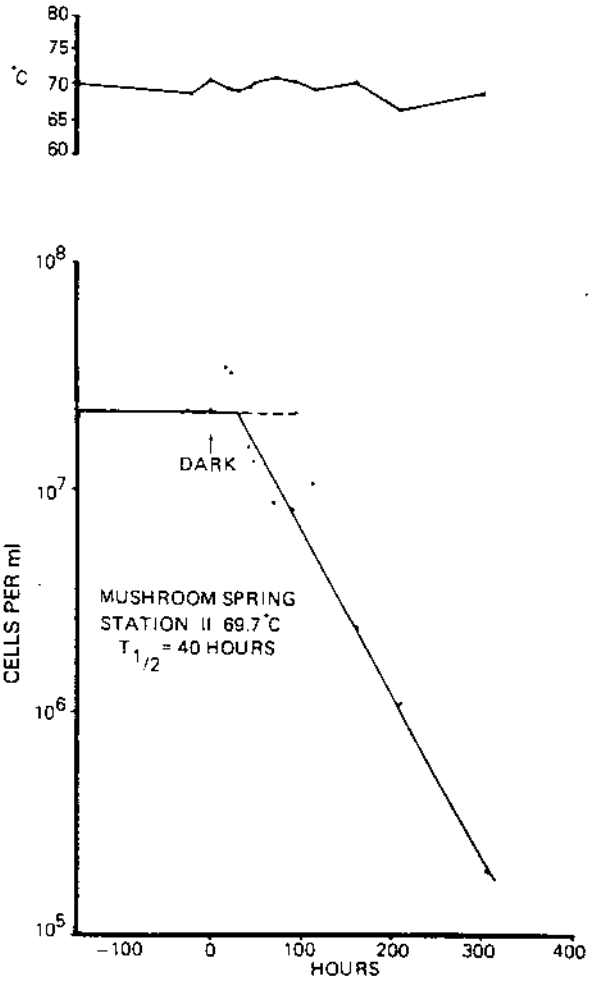


FIG. 7.10 Washout rate of part of an algal mat at a station at Mushroom Spring in Yellowstone Park, Montana, after it was shaded experimentally. (From Brock and Brock, 1968, with permission.)

where N is the final cell concentration per unit area, N_0 the initial cell concentration per unit area, Δt the time required for N_0 to multiply to N cells, and g the average doubling time (generation time). It is also assumed that the cell multiplication rate is significantly slower than the rate of attachment. Once all available space on the surface has been occupied, however, the cell population on

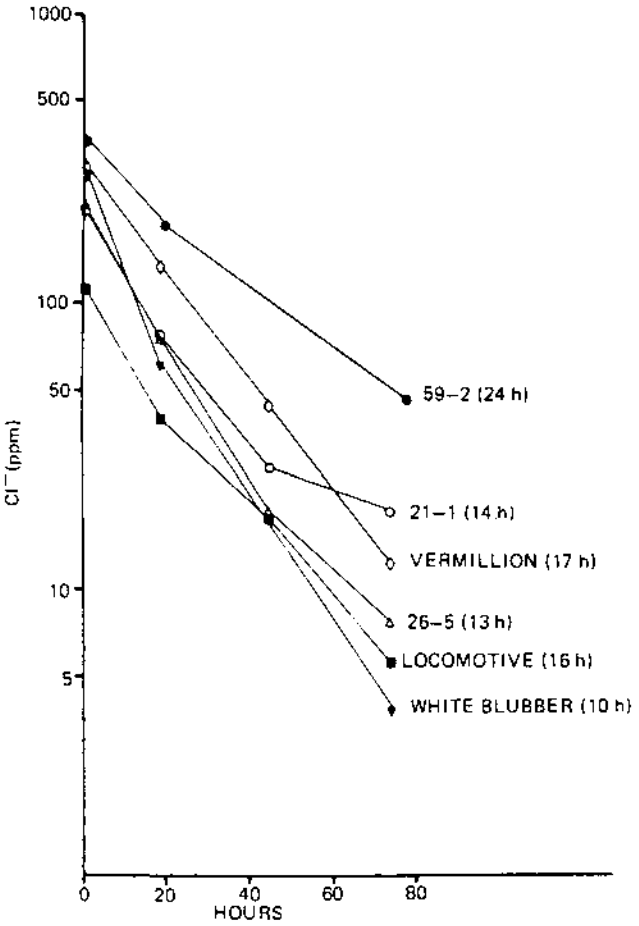


FIG. 7.11 Chloride dilution in several small springs in Yellowstone National Park, Montana. Estimated half-times for chloride dilution are given in parentheses. At site 21-1, chloride concentration had reached the natural background level by the final sampling time, and the dilution rate was estimated from the data from the first three sampling times. (From Mosser et al., 1974, with permission.)

it will remain constant (provided that the surface area does not decrease significantly due to solid substrate consumption or dissolution). The cell population in the liquid in contact with the solid will then show an arithmetic increase in cell numbers according to the relationship

$$N_{\text{final,liquid}} = N_{\text{initial,liquid}} + zN_{\text{solid}} \tag{7.6}$$

where z equals the number of cell doublings on the solid and N_{solid} equals N cells on the solid when all attachment sites are occupied. Equation (7.6) states that for every cell doubling on the surface of the cell-saturated solid, one daughter cell will be displaced into the liquid medium for lack of space on the solid. This model assumes that the liquid medium cannot support growth of the organism. As long as there is no significant change in surface area of the solid phase, the model applies. An example to which the model can be applied is the growth of autotrophic thiobacteria on water-insoluble, elemental sulfur or an appropriate metal sulfide, which serves as their sole energy source in a mineral salts solution that satisfies all other nutrient requirements (see Chaps. 18 and 19).

To introduce the element of time into Eq. (7.6), the following relationship should be considered:

$$g = \Delta t/z \tag{7.7}$$

where g represents doubling time and Δt is the time interval between $N_{\text{initial,liquid}}$ and $N_{\text{final,liquid}}$ determinations. If Eqs. (7.6) and (7.7) are combined, we get

$$N_{\text{final,liquid}} = N_{\text{initial,liquid}} + \frac{\Delta t}{g} N_{\text{solid}} \tag{7.8}$$

When solving for g, we get

$$g = \frac{\Delta t\, N_{\text{solid}}}{N_{\text{final,liquid}} - N_{\text{initial,liquid}}} \tag{7.9}$$

Espejo and Romero (1987) took a different mathematical approach to bacterial growth on a solid surface. They developed the following relationship to describe growth before surface saturation of the solid is reached:

$$\frac{dN_a}{dt} = \mu N_a \frac{N_s - N_a}{N_s} \tag{7.10}$$

where N_a is the number of attached bacteria, N_s is the limit value of bacteria that can attach to the surface under consideration, and μ is the specific growth rate. After surface saturation by the bacteria, they propose the relationship

$$\frac{dN_f}{dt} = \mu N_s \tag{7.11}$$

where N_f represents the number of free (unattached) bacteria in the liquid phase with which the surface is in contact. From Eq. (7.11), they derive the relationship

$$\mu = \frac{\Delta N_f}{\Delta t\, N_s} \tag{7.12}$$

for the specific growth rate of the culture after surface saturation. These relationships were tested by the researchers in a real experiment growing *Thiobacillus ferrooxidans* on elemental sulfur in which the sulfur was the only available energy source for the bacterium. They observed a logarithmic population increase before surface saturation and a linear increase thereafter. They concluded, however, that the value for N_s was not constant in their experiment but changed gradually after linear growth had begun.

A still different mathematical model was presented by Konishi et al. (1994), in which change in particle size of the solid substrate with time was taken into account as well as cell adsorption to the solid substrate.

7.7 TEST FOR DISTINGUISHING BETWEEN ENZYMATIC AND NONENZYMATIC GEOMICROBIAL ACTIVITY

To determine if a geomicrobial transformation is an enzymatic or a nonenzymatic process, attempts should be made to reproduce the phenomenon with cell-free extract, especially if a single organism is involved. If catalysis is observed with cell-free extract, identification of the enzyme system should be undertaken by standard techniques. Spent culture medium from which all cells have been removed, e.g., by filtration or by inactivation by heating, should also be tested for activity. If equal levels of activity are observed with untreated and treated spent medium, operation of a nonenzymatic process may be inferred. If the level of activity in treated cell-free extract is lower than in untreated cell-free spent medium, extracellular enzyme activity may be present in the untreated cell-free spent medium. This can be verified by testing activity in unheated cell-free spent medium over a range of temperatures. If a temperature optimum is observed, at least part of the activity in the cell-free medium can be attributed to extracellular enzymes.

7.8 STUDY OF REACTION PRODUCTS OF A GEOMICROBIAL TRANSFORMATION

Ideally, the products of geomicrobial transformation, if they are precipitates, should be studied not only in respect to chemical composition but also in respect to mineralogical properties through one or more of the following techniques:

scanning (SEM) and transmission (TEM) electron microscopy, including energy-dispersive X-ray measurements (EDX), microprobe examination, X-ray photoelectron spectroscopy (XPS), infrared spectroscopy, X-ray diffraction, X-ray absorption fine structure (XFAS) and X-ray absorption near-edge structure spectroscopy (XANES), and mineralogical examination with a polarizing microscope. Similar studies should ideally be undertaken on the substrate if it is an insoluble mineral or mineral complex, to be able to detect any mineralogical changes it may have undergone with time during a geomicrobial transformation.

Studies of geomicrobial phenomena require ingenuity in the application of standard microbiological, chemical, and physical techniques and often require collaboration among microbiologists, biochemists, geochemists, mineralogists, and other specialists to unravel a problem.

7.9 SUMMARY

Geomicrobial phenomena can be studied in the field and in the laboratory. Direct observation may involve microscopic examination and chemical and physical measurements. Very specific and sensitive molecular biological techniques can be applied to determine the number and kinds of microbes in field samples. Laboratory study may involve an artificial reconstruction of a geomicrobial process. Special methods have been devised for sampling, for direct observation, and for laboratory manipulation. The latter two categories include the use of fluorescence microscopy, radioactive tracers, and mass spectrometry for observing microorganisms in situ, for measuring process rates, and for measuring microbial isotope fractionation, respectively. The chemostat principle has been applied in the field to measure natural growth rates. It has also been used under simulated conditions for determining limiting substrate concentrations. Study of growth on particle surfaces may require special experimental approaches.

REFERENCES

Amann RI, Krumholz L, Stahl DA. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies. J Bacteriol 172:762–770.

Amann RI, Zarda B, Stahl DA, Schleifer KH. 1992. Identification of individual prokaryotic cells using enzyme-labeled, rRNA-targeted oligonucleotide probes. Appl Environ Microbiol 58:3007–3011.

Amann RI, Ludwig W, Schleifer K-H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169.

Barker WW, Banfield JF. 1998. Zones of chemical and physical interaction at interfaces between microbial communities and minerals: A model. Geomicrobiol J 15:223–244.

Beard BL, Johnson CM, Cox L, Sun H, Nealson KH, Aguilar C. 1999. Iron isotope biosignatures. Science 285:1889–1892.

Beeman RE, Suflita JM. 1989. Evaluation of deep subsurface sampling procedures using serendipitous microbial contaminants as tracer organisms. Geomicrobiol J 7:223–233.

Bien E, Schwartz W. 1965. Geomikrobiologische Untersuchungen. IV. Über das Vorkommen konservierter toter und lebender Bakterienzellen in Salzgesteinen. Z Allg Mikrobiol 5:185–205.

Bohlool BB, Brock TD. 1974. Immunofluorescence approach to the study of the ecology of *Thermoplasma acidophilium* in a coal refuse material. Appl Microbiol 28:11–16.

Braun-Howland EB, Danielsen SA, Nierzwicki-Bauer SA. 1992. Development of a rapid method for detecting bacterial cells in situ using 16S rRNA-targeted probes. Biotechniques 13:928–934.

Braun-Howland EB, Vescio PA, Nierzwicki-Bauer SA. 1993. Use of a simplified cell blot technique and 16S rRNA-directed probes for identification of common environmental isolates. Appl Environ Microbiol 59:3219–3224.

Brock TD. 1978. Thermophilic Microorganisms and Life at High Temperatures. New York: Springer-Verlag.

Brock TD, Brock ML. 1968. Measurement of steady-state growth rates of a thermophilic alga directly in nature. J Bacteriol 95:811–815.

Bromfield SM. 1956. Oxidation of magnanese by soil microorganisms. Aust J Biol Sci 9:238–252.

Bromfield SM, Skerman VBD. 1950. Biological oxidation of manganese in soils. Soil Sci 69:337–348.

Burdige DJ, Kepkay PE. 1983. Determination of bacterial manganese oxidation rates in sediments using an in-situ dialysis technique. I. Laboratory studies. Geochim Cosmochim Acta 47:1907–1916.

Casida LE. 1962. On the isolation and growth of individual microbial cells from soil. Can J Microbiol 8:115–119.

Casida LE. 1965. Abundant microorganisms in soil. Appl Microbiol 13:327–334.

Casida LE. 1971. Microorganisms in unamended soil as observed by various forms of microscopy and staining. Appl Microbiol 21:1040–1045.

Clesceri LS, Greenberg AE, Trussell RR, eds. 1989. Standard Methods for the Examination of Water and Wastewater. 17th ed. Washington, DC: Am Public Health Assoc, pp 9-65 to 9-66.

De La Rocha CL, Brzezinski MA, DeNiro MJ. 2000. A first look at the distribution of the stable isotopes of silicon in natural waters. Geochim Cosmochim Acta 64:2467–2477.

DeLong EF, Wickham GS, Pace NR. 1989. Phylogenetic stains: Ribosomal RNA-based probes for the identification of single cells. Science 243:1360–1363.

Dubinina GA. 1970. Untersuchungen über die Morphologie und die Beziehung zu *Mycoplasma*. Z Allg Mikrobiol 10:309–320.

Edwards KJ, Goebel BM, Rodgers TM, Schrenk MO, Gihring TM, Cardona MM, Hu B, McGuire MM, Hamers RJ, Pace NR, Banfield JF. 1999. Geomicrobiology of pyrite (FeS_2) dissolution: Case study at Iron Mountain, CA. Geomicrobiol J 16:155–179.

Emerson S, Kalhorn S, Jacobs L, Tebo BM, Nealson KH, Rosson RA. 1982. Environmental oxidation rate of manganese(II): Bacterial catalysis. Geochim Cosmochim Acta 46:1073–1079.

Emiliani C, Hudson J, Shinn A, George RY. 1978. Oxygen and carbon isotope growth record in a reef coral from the Florida Keys and a deep-sea coral from the Blake Plateau. Science 202:627–629.

Eren J, Pramer D. 1966. Application of immunofluorescent staining to studies of the ecology of soil microorganisms. Soil Sci 101:39–49.

Espejo RT, Romero P. 1987. Growth of *Thiobacillus ferrooxidans* on elemental sulfur. Appl Environ Microbiol 53:1907–1912.

Estep MF, Hoering TC. 1980. Biogeochemistry of the stable hydrogen isotopes. Geochim Cosmochim Acta 44:1197–1206.

Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GB, eds. 1981. Manual of Methods for General Bacteriology. Washington, DC: Am Soc for Microbiol.

Gerhardt P, Murray RGE, Wood WA, Krieg NR. 1993. Methods for General and Molecular Bacteriology. Washington, DC: ASM Press.

Ghiorse WC, Balkwill DL. 1983. Enumeration and morphological characterization of bacteria indigenous to subsurface environments. Dev Ind Microbiol 24:213–224.

Giovannoni SJ, DeLong EF, Olson GJ, Pace NR. 1988. Phylogenetic group-specific oligonucleotide probes for identification of single microbial cells. J Bacteriol 170:720–726.

Griffin WT, Phelps TJ, Colwell FS, Fredrickson JK. 1997. Methods for obtaining deep subsurface microbiological samples by drilling. In: Amy PS, Haldeman DL, eds. The Microbiology of the Terrestrial Deep Subsurface. Boca Raton, FL: CRC Lewis, pp 23–44.

Hoefs J. 1997. Stable Isotope Geochemistry. 4th ed. Berlin: Springer-Verlag, pp 5, 41–42, 59–60.

Hornor SG. 1984. Microbial leaching of zinc concentrate in fresh-water microcosms: Comparison between aerobic and oxygen-limited conditions. Geomicrobiol J 3:359–371.

Huber H, Huber G, Stetter KO. 1985. A modified DAPI fluorescence staining procedure suitable for the visualization of lithotrophic bacteria. Syst Appl Microbiol 6:105–106.

Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV. 1997. Manual of Environmental Microbiology. Washington, DC: ASM Press.

Ivanov MV. 1968. Microbiological Processes in the Formation of Sulfur Deposits. Published by the U.S Department of Agriculture and the National Science Foundation, Washington, DC (Israel Program for Scientific Translations).

Jannasch HW. 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol Oceanogr 12:264–271.

Jannasch HW. 1969. Estimation of bacterial growth in natural waters. J Bacteriol 99:156–160.

Jannasch HW, Mottl MJ. 1985. Geomicrobiology of the deep-sea hydrothermal vents. Science 229:717–725.

Jannasch HW, Wirsen CO. 1981. Morphological survey of microbial mats near deep-sea thermal vents. Appl Environ Microbiol 41:528–538.

Jones GE, Starkey RL. 1957. Fractionation of stable isotopes of sulfur by microorganisms and their role in deposition of native sulfur. Appl Microbiol 5:111–118.
Jones JG, Gardener S, Simon BM. 1983. Bacterial reduction of ferric iron in a stratified eutrophic lake. J Gen Microbiol 129:131–139.
Jones JG, Gardener S, Simon BM. 1984. Reduction of ferric iron by heterotrophic bacteria in lake sediment. J Gen Microbiol 130:45–51.
Jurtshuk RJ, Blick M, Bresser J, Fox GE, Jurtshuk P Jr. 1992. Rapid in situ hybridization technique using 16S rRNA segements for detecting and differentiating the closely related gram-positive organisms *Bacillus polymyxa* and *Bacillus macerans*. Appl Environ Microbiol 58:2571–2578.
Kepner RL Jr, Pratt JR. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: Past and present. Microbiol Rev 58:603–615.
Kieft TL, Caldwell DE. 1984. Weathering of calcite, pyrite, and sulfur by *Thermothrix thiopara* in a thermal spring. Geomicrobiol J 3:201–215.
Konishi YY, TakasakaY, Asai S. 1994. Kinetics of growth and elemental sulfur oxidation in batch culture of *Thiobacillus ferrooxidans*. Biotechnol Bioeng 44:667–673.
LaRock PA. 1969. The bacterial oxidation of manganese in a freshwater lake. PhD Thesis. Rensselaer Polytechnic Inst, Troy, NY.
LaRock PA, Ehrlich HL. 1975. Observations of bacterial microcolonies on the surface of ferromanganese nodules from Blake Plateau by scanning electronmicroscopy. Microb Ecol 2:84–96.
Lawrence JR, Korber DR, Wolfaardt GM, Caldwell DE. 1997. Analytical imaging and microscopy techniques. In: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV, eds. Manual of Environmental Microbiology. Washington, DC: ASM Press, pp 29–51.
Madigan MR, Martinko AJM, Parker J. 2000. Brock. Biology of Microorganisms. Upper Saddle River, NJ: Prentice-Hall, pp 432–438.
Mandernack KW, Bazylinski DA, Shanks WC III, Bullen TD. 1999. Oxygen isotope studies of magnetite produced by magnetotactic bacteria. Science 285:1892–1896.
Mortimer RJG, Coleman ML. 1997. Microbial influence on the oxygen isotopic composition of diagenetic siderite. Geochim Cosmochim Acta 61:1705–1711.
Mosser JL, Bohlol BB, Brock TD. 1974. Growth rates of *Sulfolobus acidocaldarius* in nature. J Bacteriol 118:1075–1081.
Muyzer G, de Bruyn AC, Schmedding DJM, Bos P, Westbroek P, Kuenen GJ. 1987. A combined immunofluorescence-DNA fluorescence staining technique for enumeration of *Thiobacillus ferrooxidans* in a population of acidophilic bacteria. Appl Environ Microbiol 53:660–664.
Muyzer G, de Waal EC, Uitterlinden AG. 1993. Profiling of complex microbial populations by denaturing gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700.
Pedersen K. 1993. The deep subterranean biosphere. Earth-Sci Rev 34:243–260.
Perfil'ev BV, Gabe DR. 1965. The use of the microbial-landscape method to investigate bacteria which concentrate manganese and iron in bottom deposits. In: Perfil'ev BV, Gabe DR, Gal'perina AM, Rabinovich VA, Sapotnitskii AA, Sherman EE, Troshanov EP, eds. Applied Capillary Microscopy: The Role of Microoganisms in the Formation of Iron and Manganese Deposits. New York: Consultants Bureau, pp 9–54.

Perfil'ev BV, Gabe DR. 1969. Capillary Methods for Studying Microorganisms. Engl Transl by Shewan, J. Toronto, Canada: Univ Toronto Press.

Phelps TJ, Russell BF. 1990. Drilling and coring deep subsurface sediments for microbiological investigations. In: Fliermans CB, Hazen TC, eds. Proceedings of the First International Symposium on Microbiology of the Deep Subsurface. Aiken, SC:WSRC Infor Services Sec Publ Group, pp 2-35 to 2-47.

Sakaguchi T, Tomita O. 2000. Bioseparation of lithium isotopes by using microorganisms. Resource Environ Bioechnol 3:173–182.

Sand MD, LaRock PA, Hodson RE. 1975. Radioisotope assay for the quantification of sulfate reducing bacteria in sediment and water. Appl Microbiol 29:626–634.

Saylor G, Layton AC. 1990. Environmental application of nucleic acid hybridization. Annu Rev Microbiol 44:625–648.

Schmidt EL, Bankole RO. 1965. Specificity of immunofluorescent staining for study of *Aspergilllus flavus* in soil. Appl Microbiol 13:673–679.

Schopf JW, ed. 1983. Earth's Earliest Biosphere: Its Origin and Evolution. Princeton, NJ: Princeton Univ Press.

Schweisfurth R. 1969. Mangan-oxydierende Pilze. Z Parasitenk, Infektionskr Hyg Abt 1, Orig 212:486–491.

Sieburth JMcN. 1975. Microbial Seascapes. A Pictorial Essay on Marine Microorganisms and Their Environments. Baltimore, MD: Univ Park Press.

Skerman VBD. 1967. A Guide to the Identification of the Genera of Bacteria. With Methods and Digests of Generic Characteristics. 2nd ed. Baltimore, MD: Williams and Wilkins.

Stahl DA. 1997. Molecular approaches for the measurement of density, diversity and phylogeny. In: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV, eds. Manual of Environmental Microbiology. Washington, DC: ASM Press, pp 102–114.

Tsien HC, Bratina BJ, Tsuji K, Hanson RS. 1990. Use of oligonucleotide signature probes for identification of physiological groups of methylotrophic bacteria. Appl Environ Microbiol 56:2858–2865.

Tuovila BJ, LaRock PA. 1987. Occurrence and preservation of ATP in Antarctic rocks and its implication in biomass determination. Geomicrobiol J 5:105–118.

Ward DM, Weller R, Bateson MM. 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature (Lond) 345:63–65.

Ward DM, Bateson MM, Weller R, Ruff-Roberts AL. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. Adv Microb Ecol 12:219–286.

Ward DM, Ferris MJ, Nold SC, Bateson MM. 1998. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol Mol Biol Rev 62:1353–1370.

Weirich G, Schweisfurth R. 1985. Extraction and culture of microorganisms from rock. Geomicrobiol J 4:1–20.

Wellman RP, Cook FD, Krouse HR. 1968. Nitrogen-15: Microbiological alterations in abundance. Science 161:269–270.

Zavarzin GA. 1961. Symbiotic culture of a new microorganism oxidizing manganese. Mikrobiologiya 30:393–395 (Engl transl pp 343–345).

Zavarzin GA. 1962. Symbiotic oxidation of manganese by two species of *Pseudomonas*. Mikrobiologiya 31:586–588 (Engl transl pp 481–482).

8

Microbial Formation and Degradation of Carbonates

8.1 DISTRIBUTION OF CARBON IN THE EARTH'S CRUST

Carbon is an element central to all life on Earth. Even though it is one of the less abundant elements in the crust (320 ppm) (Weast and Astle, 1982), it is widely but unevenly distributed (Fig. 8.1). In some places it occurs at high concentrations in nonliving matter. Much of the carbon on the surface of the Earth is tied up inorganically in the form of carbonates such as limestone and dolomite, amounting to approximately 1.8×10^{22} g of carbon. Much is also trapped as aged organic matter such as bitumen and kerogen and as coal, shale organic matter, natural gas, and petroleum. This carbon amounts to about 2.5×10^{22} g as compared with around 3.5×10^{18} g of carbon in unaged, dead organic matter in soils and sediments and around 8.3×10^{17} g of carbon in living matter (estimates from Fenchel and Blackburn, 1979; Bowen, 1979). The atmosphere around the Earth holds about 6.4×10^{17} g of carbon as CO_2 (Bolin, 1970; Fenchel and Blackburn, 1979). From the quantities of carbon in each of these compartments it is seen that the carbon in living matter represents only a small fraction of the total carbon, as does the carbon in unaged, dead organic matter and atmospheric carbon. The carbon in limestone and dolomite and in aged organic

matter, insofar as it is not mined as fossil fuel and combusted by human beings, is not readily available for assimilation by living organisms. Therefore, living systems have to depend on unaged, dead organic matter and atmospheric carbon. In order not to exhaust this carbon, it has to be recycled by biological mineralization of organic matter (see Chap. 6). In the recycling process, some of the carbon enters the atmosphere as CO_2, some is trapped in carbonate deposits, and some is reassimilated by the living organisms. In the absence of human interference, **homeostasis** is assumed to operate insofar as transfer of carbon among compartments representing living and dead organic matter and the atmosphere is concerned. Present fears are that human interference is increasing the size of the atmospheric reservoir of carbon through the combustion of fossil fuels because the remaining reservoirs cannot accommodate the extra CO_2 from this combustion. The consequence of this CO_2 buildup in the atmosphere is blockage of heat radiation into space and an overall warming of the Earth's climate.

8.2 BIOLOGICAL CARBONATE DEPOSITION

Some of the CO_2 generated in biological respiration can be fixed in insoluble carbonates. Indeed, a significant portion of the insoluble carbonate at the Earth's surface is of biogenic origin, but another portion is the result of magmatic and metamorphic activity (see, e.g., Bonatti, 1966; Skirrow, 1975; Berg, 1986). Biological fixation of carbon in carbonates involves some bacteria, fungi, and algae as well as some metazoa. The carbonates can be deposited extra- and intracellularly. The bacteria, including cyanobacteria, as well as some fungi that are involved, deposit calcium carbonate extracellularly (Bavendamm, 1932; Monty, 1972; Krumbein, 1974, 1979; Morita, 1980; Verrecchia et al., 1990; Chafetz and Buczynski, 1992). The bacterium *Achromatium oxaliferum* seems to be an exception. It has been reported to deposit calcium carbonate intracellularly (Buchanan and Gibbons, 1974; De Boer et al., 1971). Some algae, including certain green, brown, and red algae, and chrysophytes such as coccolithophores (Lewin, 1965) deposit calcium carbonate as surface structures of their cells, and some protozoa lay it down as tests or shells (foraminifera). Calcium carbonate is also incorporated into skeletal support structures of certain sponges and invertebrates such as coelenterates (corals), echinoderms, bryozoans, brachiopods, and mollusks. In arthropods it is associated with their chitinous exoskeleton. The function of the structural calcium carbonate in each of these organisms is to serve as support and/or protection. In all these cases, calcium and some magnesium ions are combined with carbonate ions of biogenic origin (Lewin, 1965). Figure 8.2 illustrates a massive biogenic carbonate deposit in the form of chalk, the White Cliffs of Dover, England.

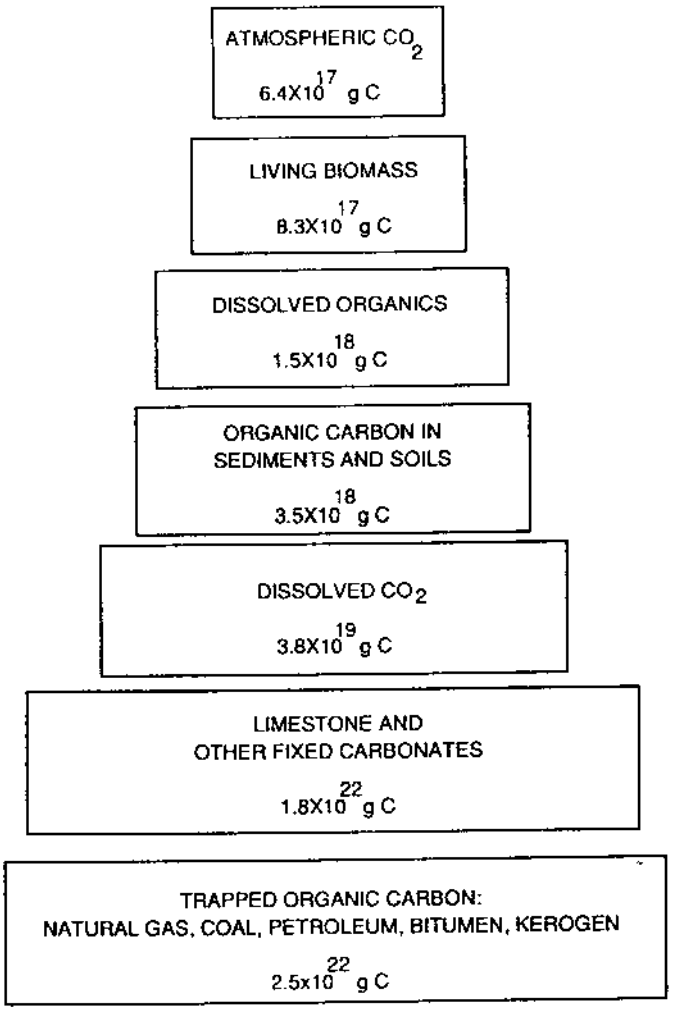


FIG. 8.1 Distribution of carbon in the lithosphere of the Earth. (Estimates from Fenchel and Blackburn, 1979; Bowen, 1979.)

Historical Perspective of the Study of Carbonate Deposition

The beginnings of the study of microbial carbonate precipitation go back to the late nineteenth century when Murray and Irvine (1889/1890) and Steinmann (1899/1901) reported formation of $CaCO_3$ in conjunction with urea decomposi-

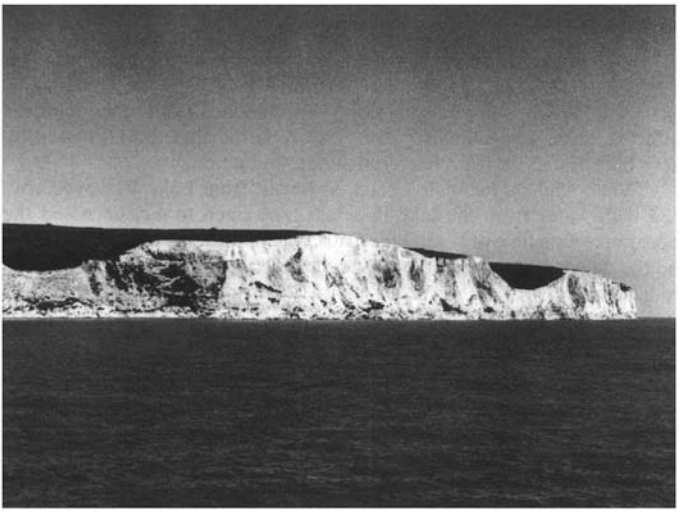


FIG. 8.2 White Cliffs of Dover, England; a foraminiferal chalk deposit. (Courtesy of the British Tourist Authority, New York, NY.)

tion and putrefaction in seawater culture media caused by microbial activity (as cited by Bavendamm, 1932). These investigations and others have elicited some controversy.

In 1899 and 1903, G. A. Nadson (see Nadson, 1903; 1928) presented the first extensive evidence that bacteria could precipitate $CaCO_3$. Nadson studied the process in Lake Veisovoe in Kharkov, southern Ukraine. He described this lake as shallow with a funnel-like deepening to 18 m near its center and as resembling the Black Sea physicochemically and biologically. He found the lake bottom to be covered by black sediment with a slight admixture of calcium carbonate. He also noted that the lake water contained $CaSO_4$, with which it was saturated at the bottom. He found that in winter the lake water was clear to a depth of 15 m. From there to the bottom it was turbid, owing to a suspension of elemental sulfur (S^0) and $CaCO_3$. The clear water revealed a varied flora and fauna and a microbial population resembling that of the Black Sea. Gorlenko et al. (1974) demonstrated that a significant part of the sulfur was the result of H_2S oxidation by photosynthetic bacteria, especially the green sulfur bacterium *Pelodictyon phaeum*, along with thiobacilli and other colorless sulfur bacteria. These investigators also reported that the H_2S used by these bacteria arises from bacterial sulfate reduction.

When Nadson incubated black sediment from Lake Veisovoe covered with water from the lake in a test tube open to the air, he noted the appearance with

time of crystalline $CaCO_3$ in the water above the sediment. He also noted the development of a rust color on the sediment surface due to oxidized iron (Fig. 8.3). These changes did not occur with sterilized sediment unless it was inoculated with a small portion of unsterilized sediment. When access of air was blocked after development of $CaCO_3$ and ferric oxide and the test tube was then reincubated, the reactions were reversed. The $CaCO_3$ dissolved, and the sediment again turned black (Fig. 8.3). Nadson isolated a number of organisms from the black lake sediment, including *Proteus vulgaris*, *Bacillus mycoides*, *B. salinus* n.sp., *Actinomyces albus* Gasper., *A. verrucosis* n.sp., and *A. roseolus* n.sp. He did not report the presence of sulfate-reducing bacteria, which were still unknown at that time.

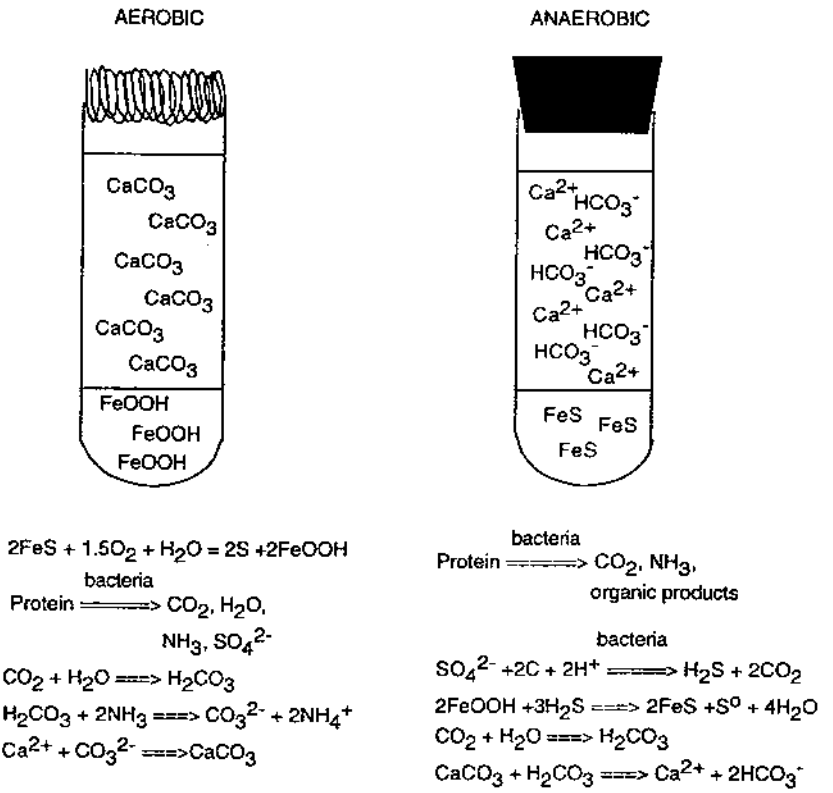


FIG. 8.3 Diagrammatic representation of Nadson's experiments with mud from Lake Veisovoe. Contents of the tubes represent the final chemical state after microbial development. Chemical equations describe important reactions leading to the final chemical state.

When in a separate experiment Nadson incubated a pure culture of *P. vulgaris* in a test tube containing sterilized, dried lake sediment and a 2% peptone solution prepared in distilled water, he noted the development of a pellicle of silicic acid that yellowed in time and then became brown and opaque from ferric hydroxide deposition. At the same time he noted that $CaCO_3$ was deposited in the pellicle. None of these changes occurred in sterile, uninoculated medium.

In other experiments, Nadson observed $CaCO_3$ formation by *B. mycoides* in nutrient broth, nutrient agar, and gelatin medium. He did not identify the source of the calcium in these instances, but it must have been a component of each of the media. He also noted that bacterial decomposition of dead algae and invertebrates in seawater led to the precipitation of $CaCO_3$. He further reported the formation of ooliths (i.e., shell-like deposits around normal and involuted cells) by *Bacterium helgolandium* in 1% peptone seawater broth. He had isolated the bacterium from decomposing *Alcyonidium*, an alga. Lastly, Nadson claimed to have observed precipitation of magnesian calcite or dolomite in two experiments. One involved three and a half years of incubation of black mud from a salt lake near Kharkov. The second involved one and a half years of incubation of a culture of *P. vulgaris* growing in a mixture of sterilized sediment from Lake Veisovoe and seawater enriched with 2% peptone.

Nadson's pioneering experiments showed that $CaCO_3$ precipitation was not brought about by any special group of bacteria but depended on appropriate conditions. On the other hand, Drew (1911, 1914), who was apparently not aware of Nadson's studies, concluded from laboratory experiments that in the tropical seas around the Bahamas, denitrifying bacteria were responsible for $CaCO_3$ precipitation. He found that water samples from this geographical location contained denitrifying bacteria in significant numbers. He isolated a denitrifying, $CaCO_3$-precipitating culture from the water samples, which he named *Bacterium calcis*. He apparently held it responsible for the $CaCO_3$ precipitation in the sea (see Bavendamm, 1932). Although many geologists of his day appeared to accept Drew's explanation of the origin of the $CaCO_3$ in the seas around the Bahamas, it is not accepted today.

Lipman (1929) rejected Drew's conclusion on the basis of some studies of waters off the island of Tutuila (American Samoa) in the Pacific Ocean and the Tortugas in the Gulf of Mexico. In his work, Lipman confined himself mainly to an examination of water samples and only a few sediment samples. Using various culture media, he was able to demonstrate $CaCO_3$ precipitation in the laboratory by a variety of bacteria, not just *B. calcis*, as Drew had suggested. However, because the number of viable organisms in the water samples and in the few sediment samples that Lipman examined seemed small to him, he felt that these organisms were not important in $CaCO_3$ precipitation in the sea. He probably should have examined the sediments more extensively.

Bavendamm (1932) reintroduced Nadson's concept of microbial $CaCO_3$ precipitation as an important geochemical process that is not a specific activity of any special group of bacteria. As a result of his microbiological investigations in the Bahamas, he concluded that this precipitation occurs chiefly in sediments of shallow bays, lagoons, and swamps. He isolated and enumerated heterotrophic and autotrophic bacteria, including sulfur bacteria, photosynthetic bacteria, agar-, cellulose-, and urea-hydrolyzing bacteria, nitrogen-fixing bacteria, and sulfate-reducing bacteria. According to him, all of these had an ability to precipitate $CaCO_3$. He rejected the idea of significant participation of cyanobacteria in $CaCO_3$ precipitation. As we know now, however, some cyanobacteria can cause significant $CaCO_3$ deposition (Golubic, 1973).

There is still no complete consensus about the origin of the calcium carbonate suspended in the seas around the Bahamas. Some geochemists favor an inorganic mechanism of formation (Skirrow, 1975). They feel that a finding of supersaturation with $CaCO_3$ of the waters around the Bahamas and an abundant presence of nuclei for $CaCO_3$ deposition explains the $CaCO_3$ precipitation in the simplest way. Others, such as Lowenstamm and Epstein (1957), favor algal involvement based on a study of $^{18}O/^{16}O$ ratios of the precipitated carbonates. McCallum and Guhathakurta (1970) isolated a number of different bacteria from sediments from Bimini, Brown's Cay, and Andros Island in the Bahamas, which had a capacity to precipitate calcium carbonate in most cases as aragonite under laboratory conditions. They concluded that the naturally formed calcium carbonate in the Bahamas is the result of a combination of biological and chemical factors. This also appears from the work of Buczynski and Chafetz (1991), who illustrated in elegant scanning electron photomicrographs the ability of marine bacteria to induce calcium carbonate precipitation in the form of calcite and aragonite (Fig. 8.4). Very recent investigations of the origin of the calcium carbonate suspended in the waters around the Great Bahama Banks west of Andros Island suggest coupling on a biophysicochemical level between the microbial community, physical circulation on the Bank, and water chemistry, but further study is needed to fully resolve the phenomenon (Thompson, 2000).

Despite the now numerous reports of calcium carbonate precipitation by bacteria other than cyanobacteria (e.g., Krumbein, 1974,1979; Shinano and Sakai, 1969; Shinano, 1972a,b; Ashirov and Sazonova, 1962; Greenfield 1963; Abd-el-Malek and Rizk, 1963a; Roemer and Schwartz, 1965; McCallum and Guhathakurta, 1970; Morita, 1980; del Moral et al., 1987; Ferrer et al., 1988a,b; Thompson and Ferris, 1990; Rivadeneyra et al., 1991; Buczynski and Chafetz, 1991), the significance of many of these reports has been questioned by Novitsky (1981). He feels that in most instances, in situ environmental conditions do not meet the requirements (especially pH values > 8.3) essential for calcium carbonate precipitation in laboratory experiments. He was unable to demonstrate

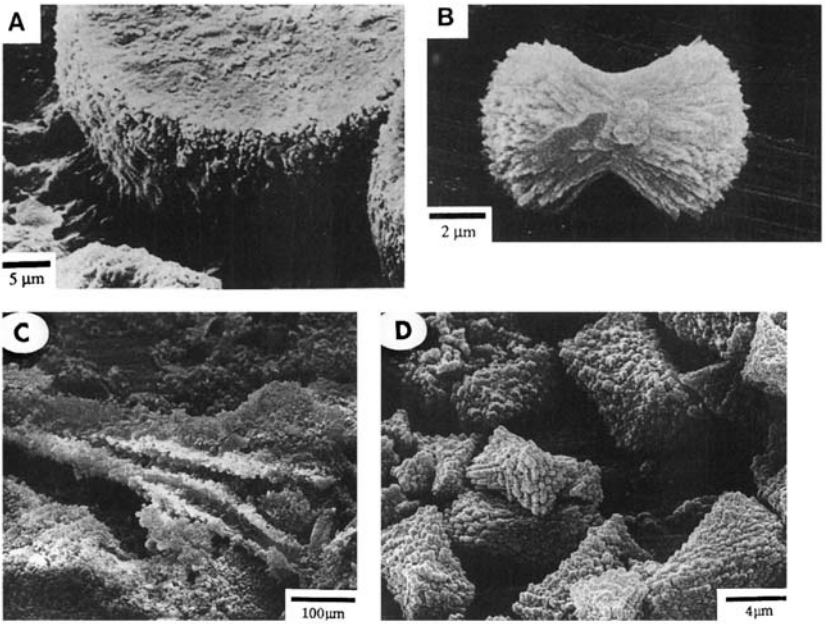


FIG. 8.4 Bacterial precipitation of calcium carbonate as aragonite and calcite. Scanning electron micrographs of (A) a crust of a hemisphere of aragonite precipitate formed by bacteria in liquid culture in the laboratory; (B) an aragonite dumbbell precipitated by bacteria in liquid medium in the laboratory; (C) crystal bundles of calcite encrusting dead cyanobacterial filaments that were placed in gelatinous medium inoculated with bacteria from Baffin Bay. The specimen was treated with 30% H_2O_2 to remove organic matter. It should be noted that the crystal aggregates have been cemented to form a rigid crust that does not depend on the organic matter for support. (D) Higher magnification view of some of the crystal aggregates from (C) [area close-up not from field of view in (C)]. These aggregates sometimes resemble rhombohedra, tetragonal disphenoids, or tetragonal pyramids. [(A) and (B) from Buczinski and Chafetz, 1991, with permission; (C) and (D) from Chafetz and Buczinski, 1992, with permission.]

$CaCO_3$ precipitation with active laboratory isolates at in situ pH in water samples from around Bermuda and from the Halifax, Nova Scotia, harbor. Novitsky did not, however, acknowledge the laboratory observation by McCallum and Guhathakurta (1970) of bacterial $CaCO_3$ precipitation by a number of different organisms in a calcium acetate-containing seawater medium with added KNO_3 in which the pH fell to about 6.0. The authors of this study stressed that the mineral form of the calcium carbonate that precipitated in the presence of the bacteria was aragonite, as is generally observed in situ in marine environments. When calcium

TABLE 8.1 Solubility Products of Some Carbonates

Compound	Solubility constant (K_{sol})	Ref.
$CaCO_3$	$10^{-8.32}$	Latimer and Hildebrand (1942)[a]
$MgCO_3 \cdot 3H_2O$	10^{-5}	Latimer and Hildebrand (1942)
$MgCO_3$	$10^{-4.59}$	Weast and Astle (1982)
$CaMg(CO_3)_2$	$10^{-16.7}$	Stumm and Morgan (1981)

[a] Note that Stumm and Morgan (1981) give a solubility product in fresh water of $10^{-8.42}$ (25°C) for calcite and $10^{-8.22}$ (25°C) for aragonite.

carbonate precipitated in any of their sterile controls, it seemed to be in a different mineral form.

Basis for Microbial Carbonate Deposition

Carbonate compounds are relatively insoluble. Table 8.1 lists the solubility constants for several geologically important carbonates. Because of the relative insolubility of the carbonates, they are readily precipitated from solution at relatively low carbonate and counter-ion concentrations. The following will illustrate the fact.

In a solution containing $10^{-4.16}$ M Ca^{2+},* the calcium will be precipitated by CO_3^{2-} at a concentration in excess of $10^{-4.16}$ M. This is because the product of the concentrations of the two ions will exceed the solubility product of $CaCO_3$, which is

$$[Ca^{2+}][CO_3^{2-}] = K_{sol} = 10^{-8.32} \tag{8.1}$$

In general, Ca^{2+} will be precipitated as $CaCO_3$ when the carbonate ion concentration is in excess of the ratio $10^{-8.32}/[Ca^{2+}]$ [see Eq. (8.1)].

Carbonate ion in an unbuffered aqueous solution undergoes hydrolysis. This process causes a solution of 0.1 M Na_2CO_3 to develop a hydroxyl ion concentration of 0.004 M. The following reactions explain this phenomenon.

$$Na_2CO_3 \Leftrightarrow 2Na^+ + CO_3^{2-} \tag{8.2}$$

$$CO_3^{2-} + H_2O \Leftrightarrow HCO_3^- + OH^- \tag{8.3}$$

$$HCO_3^- + H_2O \Leftrightarrow H_2CO_3 + OH^- \tag{8.4}$$

Of these three reactions, the third can be considered negligible.

* The ion concentrations here refer really to ion activities.

Since bicarbonate dissociates according to the reaction

$$HCO_3^- \Leftrightarrow CO_3^{2-} + H^+ \tag{8.5}$$

whose dissociation constant (K_2) is

$$\frac{[CO_3^{2-}][H^+]}{[HCO_3^-]} = 10^{-10.33} \tag{8.6}$$

and since the ionization constant for water (K_w) at 25 °C is

$$[H^+][OH^-] = 10^{-14} \tag{8.7}$$

the equilibrium constant ($K_{equilib}$) for the hydrolysis of CO_3^{2-} [reaction (8.3)] is

$$\frac{[HCO_3^-][OH^-]}{[CO_3^{2-}]} = \frac{10^{-14}}{10^{-10.33}} = 10^{-3.67} \tag{8.8}$$

At pH 7.0, where the hydroxyl concentration is 10^{-7} [see Eq. (8.7)], the ratio of bicarbonate to carbonate is therefore

$$\frac{[HCO_3^-]}{[CO_3^{2-}]} = \frac{10^{-3.67}}{10^{-7}} = 10^{3.33} \tag{8.9}$$

This means that at pH 7.0 the bicarbonate concentration is $10^{3.33}$ times greater than the carbonate concentration in an aqueous solution, assuming an equilibrium exists between the CO_2 of the atmosphere in contact with the solution and CO_2 in solution.

From Eq. (8.1) we can predict that at a freshwater concentration of 0.03 g of Ca^{2+} per liter of solution (i.e., at $10^{-3.14}$ M Ca^{2+}), an excess of $10^{-5.18}$ M carbonate ion would be required to precipitate the calcium as calcium carbonate, because from Eq. (8.1) it follows that

$$[CO_3^{2-}] = \frac{10^{-8.32}}{10^{-3.14}} = 10^{-5.18} \tag{8.10}$$

Now, at pH 7.0, $10^{-5.18}$ M carbonate would be in equilibrium with $10^{-5.18}$ multiplied by $10^{3.33}$, equaling $10^{-1.85}$ M HCO_3^- according to Eq. (8.9). This amount of bicarbonate plus carbonate is equivalent to approximately 0.6 g of CO_2 per liter of solution.

Similarly, from Eq. (8.1) we can predict that at the calcium ion concentration in normal seawater, which is 10^{-2} M, a carbonate concentration in excess of $10^{-6.32}$ M would be required to precipitate it. At pH 8, this amount of carbonate ion would be expected to be in equilibrium with approximately $10^{-3.99}$ M bicarbonate ion, which is equivalent to about 0.0045 g of CO_2 per liter of solution. Assuming the combined concentration of carbonate and bicarbonate ions in seawater to be 2.8×10^4 μg of carbon per liter (Marine Chemistry, 1971),

we can calculate that the carbonate concentration at pH 8.0 must be about $10^{-4.97}$ M and the bicarbonate concentration about $10^{-2.64}$ M. Since the product of the carbonate concentration ($10^{-4.97}$ M) and that of the calcium concentration (10^{-2} M) is $10^{-6.97}$, which is greater than the solubility product for $CaCO_3$ ($10^{-8.32}$), seawater is saturated with respect to calcium carbonate. In reality, this seems to be true only for marine surface waters (Schmalz, 1972). (Mg^{2+} ion in seawater is not readily precipitated as $MgCO_3$ because of the relatively high solubility of $MgCO_3$.)

A quantity of 0.6 g of CO_2 can be derived from the complete oxidation of 0.41 g of glucose according to the equation

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \tag{8.11}$$

Similarly, 0.0045 g of CO_2 can be derived from the complete oxidation of 0.003 g of glucose. Such quantities of glucose are readily oxidized in a relatively short time by appropriate populations of bacteria or fungi.

Conditions for Extracellular Microbial Carbonate Precipitation

As already mentioned, some bacteria, including cyanobacteria, and fungi precipitate $CaCO_3$ or other insoluble carbonates extracellularly under various conditions. Let us define the conditions under which this can take place. They include:

1. *Aerobic and anaerobic oxidation of carbon compounds consisting of carbon and hydrogen with or without oxygen, for example carbohydrates, organic acids, and hydrocarbons*. If such oxidations occur in a well-buffered neutral or alkaline environment containing adequate amounts of calcium or other appropriate cations, at least some of the CO_2 that is generated will be transformed into carbonate, which will then precipitate with appropriate cations.

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \tag{8.12}$$

$$H_2CO_3 + OH^- \Leftrightarrow HCO_3^- + H_2O \tag{8.13}$$

$$HCO_3^- + OH^- \Leftrightarrow CO_3^{2-} + H_2O \tag{8.14}$$

Calcium carbonate precipitation under these conditions has been illustrated by the formation of aragonite and other calcium carbonates by bacteria and fungi in seawater media containing organic matter at concentrations of 0.01 and 0.1% (Krumbein, 1974). The organic matter in different experiments consisted of glucose, sodium acetate, or sodium lactate. The aragonite precipitated on the surface of the bacteria or fungi after 36 hr of incubation. Between 36 and 90 hr, the cells in the

$CaCO_3$ precipitate were still viable (although deformed), but after 4–7 days they were nonviable. Phosphate above a critical concentration can interfere with calcite formation by soil bacteria (Rivadeneyra et al., 1985).

Verrecchia et al. (1990) suggested that in semiarid regions the role of fungi is to immobilize Ca^{2+} ion with oxalate, which is a product of their metabolism. Upon death of the fungi, bacteria convert the calcium oxalate (whewellite) to secondary calcium carbonate by mineralizing the oxalate.

2. *Aerobic or anaerobic oxidation of organic nitrogen compounds with the release of NH_3 and CO_2 in unbuffered environments containing sufficient amounts of calcium, magnesium, or other appropriate cations.* The NH_3 is formed in the deamination of amines, amino acids, purines, pyrimidines, and other nitrogen-containing compounds, especially by bacteria. In water, the NH_3 hydrolyzes to NH_4OH, which dissociates partially to NH_4^+ and OH^-, thereby raising the pH of the environment to the point where at least some of the CO_2 produced may be transformed into carbonate. $CaCO_3$ precipitation under these conditions has been observed by the formation of aragonite and other calcium carbonates by bacteria and fungi in seawater media containing nutrients such as asparagine or peptone in a concentration of 0.01–0.1% or homogenized cyanobacteria (Krumbein, 1974). Moderately halophilic bacteria have been shown to precipitate $CaCO_3$ under laboratory conditions as calcite, aragonite, or vaterite, depending on culture conditions (del Moral et al., 1987; Ferrer et al., 1988a; Rivadeneyra et al., 1991). Other examples are the precipitation of calcium carbonate by various species of *Micrococcus* and a gram-negative rod in peptone media made up in natural seawater and in Lyman's artificial seawater (Shinano and Sakai, 1969; Shinano, 1972a,b). The organisms in this case came from inland seas of the North Pacific and from the western Indian Ocean. Lithification of beachrock along the shores of the Gulf of Aqaba (Sinai) is an example of in situ formation of $CaCO_3$ by heterotrophic bacteria in their mineralization of cyanobacterial remains (Krumbein, 1979).
3. *The reduction of $CaCO_4$ to CaS by sulfate-reducing bacteria such as Desulfovibrio spp., Desulfotomaculum spp., and others using organic carbon.* For the purpose of this discussion, organic carbon is indicated by the formula (CH_2O) in Eq. (8.15). It serves as their source of reducing power. The CaS formed by these organisms hydrolyzes readily to H_2S, which has a small dissociation constant ($K_1 = 10^{-6.95}$; $K_2 = 10^{-15}$). The Ca^{2+} then reacts with CO_3^{2-} derived from the CO_2 produced in the oxidation of the organic matter by the

sulfate-reducing bacteria. A reaction sequence describing the reduction of $CaSO_4$ to H_2S and $CaCO_3$ may be written as follows:

$$CaSO_4 + 2(CH_2O) \xrightarrow[\text{bacteria}]{\text{sulfate-reducing}} CaS + 2CO_2 + 2H_2O \quad (8.15)$$

$$CaS + 2H_2O \rightarrow Ca(OH)_2 + H_2S \quad (8.16)$$

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (8.17)$$

$$Ca(OH)_2 + H_2CO_3 \rightarrow CaCO_3 + 2H_2O \quad (8.18)$$

It should be noted that in reaction (8.15) as written, 2 moles of CO_2 are formed for every mole of SO_4^{2-} reduced, yet, only 1 mole of CO_2 is required to precipitate the Ca. Hence, $CaCO_3$ precipitation under these circumstances depends on one of three conditions, namely the loss CO_2 from the environment, the presence of a suitable buffer system, or the development of alkaline conditions.

Evidence for $CaCO_3$ deposition linked to bacterial sulfate reduction is found in the work of Abd-el-Malek and Rizk (1963a). They demonstrated the formation of $CaCO_3$ during bacterial sulfate reduction in experiments using fertile clay-loam soil enriched with starch and sulfate or sandy soil enriched with sulfate and plant matter. Other evidence of microbial carbonate formation during sulfate reduction is found in the work of Ashirov and Sazonova (1962) and Roemer and Schwartz (1965). Ashirov and Sazonova showed that secondary calcite was deposited when an enrichment of sulfate-reducing bacteria was grown in quartz sand bathed in Shturm's medium: $(NH_4)SO_4$, 4 g; $NaHPO_4$, 3.5 g; KH_2PO_4, 1.5 g; $CaSO_4$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; NaCl, 20 g, $(NH_4)2Fe(SO_4)_2 \cdot 6H_2O$, 0.5 g; Na_2S, 0.030 g; $NaHCO_3$, 0.5 g; distilled water, 1 L. The hydrogen (electron) donor was gaseous hydrogen (H_2), calcium lactate plus acetate (the acetate probably acted as a carbon source), or petroleum. Petroleum may have first been broken down to usable hydrogen donors for sulfate reduction by other organisms in the mixed culture (e.g., Nazina et al., 1985), which the investigators used as inoculum, before the sulfate reducers carried out their activity. The results from these experiments have lent support to the notion that incidents of sealing of some oil deposits by $CaCO_3$ may be due to the activity of sulfate-reducing bacteria at the petroleum/water interface of an oil reservoir.

Roemer and Schwartz (1965) showed that sulfate reducers were able to form calcite ($CaCO_3$) from gypsum ($CaSO_4 \cdot 2H_2O$) and from anhydrite ($CaSO_4$). Their cultures also formed strontianite ($SrCO_3$) from celestite ($SrSO_4$) and witherite ($BaO \cdot CO_2$) from barite ($BaSO_4$),

but they formed aluminum hydroxide rather than aluminum carbonate from aluminum sulfate.

Still another example of calcium carbonate formation as a result of bacterial sulfate reduction is the deposition of secondary calcite in cap rock of salt domes. This activity has been inferred from a study of $^{13}C/^{12}C$ ratios of samples taken from these deposits (Feeley and Kulp, 1957) (see also Chap. 18).

4. *The hydrolysis of urea leading to the formation of ammonium carbonate.* Urea hydrolysis can be summarized by the equation

$$NH_2(CO)NH_2 + 2H_2O \rightarrow (NH_4)_2CO_3 \tag{8.19}$$

This reaction causes precipitation of Ca^{2+}, Mg^{2+}, or other appropriate cations when present at suitable concentrations. Urea is an excretory product of ureotelic animals, including adult amphibians and mammals. This hydrolytic reaction was first observed in experiments by Murray and Irvine (see Bavendamm, 1932). They believed it to be important in the marine environment. However, they did not implicate bacteria in urea hydrolysis, whereas Steinmann (1899/1901), working independently, did (as cited by Bavendamm, 1932). Bavendamm (1932) observed extensive $CaCO_3$ precipitation by urea-hydrolyzing bacteria from the Bahamas. Most recently, Fujita et al. (2000) demonstrated the presence of urea-hydrolyzing organisms in groundwater samples from the Eastern Snake River Plain (USA) that precipitated calcite rapidly in a medium containing urea and calcium. Of three isolates, two belonged to the genus *Pseudomonas* and one to the genus *Variovorax*.

As it is now perceived, urea hydrolysis is the least important mechanism of microbial carbonate deposition in nature because urea is not a widely distributed compound.

5. *Removal of CO_2 from a biocarbonate-containing solution.* Such removal will cause an increase in carbonate ion concentration according to the relationship

$$2HCO_3^- \Leftrightarrow CO_2 + H_2O + {CO_3}^{2-} \tag{8.20}$$

In the presence of an adequate supply of Ca^{2+}, $CaCO_3$ will precipitate.

An important process of CO_2 removal is photosynthesis, in which CO_2 is assimilated as the chief source of carbon for the photosynthesizing organism. Some chemolithotrophs, as long as they do not generate acids in the oxidation of their inorganic energy source, can also promote $CaCO_3$ precipitation through assimilation of CO_2 as their sole carbon source. Examples of microbial organisms that precipitate $CaCO_3$ around them as a result of their photosynthetic

activity include certain filamentous cyanobacteria associated with stromatolites (Monty, 1972; Golubic, 1973; Walter, 1976; Krumbein and Giele, 1979; Wharton et al., 1982; Nekrasova et al., 1984) (also see below). In Flathead Lake delta, Montana, cyanobacteria and algae deposit calcareous nodules and crusts on subaqueous levees. The calcium carbonate deposition in the outer portions of the nodules and concretions may result in a local rise in pH, which promotes the dissolution of silica of diatom frustules also found on the nodules and concretions. The dissolved silica is reprecipitated with calcium carbonate in interior zones of these structures. The source of the calcium and organic carbon from which CO_2 is generated by mineralization is not the lake, which is oligotrophic, but the Flathead River feeding into the lake at the site of deposition. Deposition of the concretions and crusts coincides with periods of high productivity (Moore, 1983).

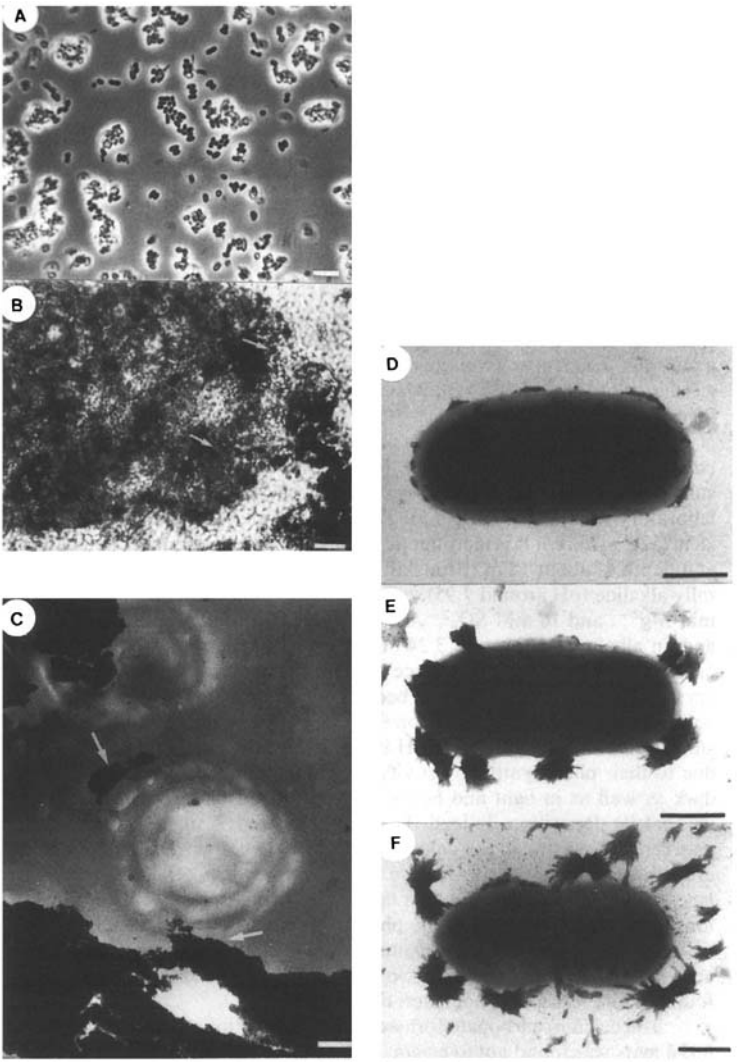
This mechanism can also function in the deposition of structural carbonate. Examples are found among some of the green, brown, and red algae and among some of the chrysophytes, which are all known to deposit calcium carbonate in their walls (e.g., Lewin, 1965; Friedmann et al., 1972). Not all calcareous algae form their $CaCO_3$ as a result of photosynthetic removal of CO_2, however; some form it from respiratory CO_2. In any case, photosynthetic removal of CO_2 is probably one of the most important mechanisms of biogenic $CaCO_3$ formation in the open, aerobic environment.

Carbonate Deposition by Cyanobacteria

Carbonate deposition by cyanobacteria has been described by Golubic (1973), Pentecost (1978), and Pentecost and Bauld (1988), among others. In this process a distinction must be made between cyanobacteria that entrap and agglutinate *preformed* calcium carbonate in their thalli and those that precipitate it in their thalli as a result of their photosynthetic activity. Preformed calcium carbonate used in entrapment and agglutination processes is formed at a site other than the site of deposition, whereas the calcium carbonate deposited as a result of photosynthetic activity of the cyanobacteria is being formed at the site of deposition (e.g., Krumbein and Potts, 1979; Pentecost and Bauld, 1988). Calcium carbonate associated with stromatolite structures (special types of cyanobacterial mats) may be a result of deposition by entrapment and agglutination or of deposition by cyanobacterial photosynthesis. In the cases of *Homoeothrix crustacea* (Pentecost, 1988) and *Lyngbya aestuarii* and *Scytonema myochrous*, it is due to photosynthesis (Pentecost and Bauld, 1988).

Calcium carbonate associated with travertine and with lacustrine carbonate crusts and nodules can result from photosynthetic activity of cyanobacteria in

freshwater environments. Travertine, a porous limestone, is formed from rapid calcium carbonate precipitation resulting, in part, from cyanobacterial photosynthesis in waterfalls and streambeds of fast-flowing rivers, in which cyanobacteria tend to be buried. The cyanobacteria avoid being trapped by outward growth or movement, which contributes to the porosity of the deposit (Golubic, 1973). On the basis of $^{14}CO_2$ photoassimilation, Pentecost (1978,1995) has



estimated that the contribution of cyanobacteria to the calcification process in travertine formation may amount to no more than about 10%, the rest of the $CaCO_3$ being formed abiotically as a result of degassing of stream water (loss of CO_2 to the atmosphere). At Waterfall Beck in Yorkshire, England, Spiro and Pentecost (1991) observed that the calcite deposited by cyanobacteria was richer in ^{13}C than nearby travertine, suggesting that the travertine was formed by a different mechanism than the calcite that formed on the cyanobacteria. Degassing of CO_2 from the streamwater may have played a role in that case of travertine formation (Pentecost and Spiro, 1990).

Lacustrine carbonate crusts are formed by benthic cyanobacteria attached to rocks or sediment, which deposit calcium carbonate through their photosynthetic activity in shallow portions of lakes with carbonate-saturated waters (Golubic, 1973). Calcareous nodules are formed around rounded rocks and pebbles or shells to which calcium carbonate-depositing cyanobacteria are attached. The nodules are rolled by water currents, thus exposing different parts of their surface to sunlight at different times and promoting photosynthetic activity and calcium carbonate precipitation by the attached cyanobacteria (Golubic, 1973). High magnesium calcite precipitated in the sheaths of certain cyanobacteria such as *Scytonema* may be related to the ability of the sheaths to concentrate magnesium three to five times over magnesium concentrations in seawater (Monty, 1967; see also discussion by Golubic, 1973).

Thompson and Ferris (1990) demonstrated the ability of *Synechococcus* from Green Lake, Lafayette, NY, to precipitate calcite, gypsum, and probably magnesite from filter-sterilized water from the lake in laboratory simulations (Fig. 8.5). The lake has an average depth of about 28 m (52.5 m maximum). It is meromictic with a distinct, permanent chemocline at a depth of about 18 m

FIG. 8.5 Calcite and gypsum precipitation by *Synechococcus* sp. isolated from Fayetteville Green Lake, New York. (A) Phase contrast photomicrograph of *Synechococcus* in laboratory culture. (B) Petrographic thin-section photomicrograph of calcite crystal from Green Lake showing evidence of occlusion of numerous small bacterial cells within calcite grain (arrows). Note similar size of *Synechococcus* in (A) and occluded bacterial cells in (B). Scale bars in (A) and (B) equal 5 µm. (C) Thin-section transmission electron micrograph of two *Synechococcus* cells and calcite from a 72 hr culture (cell represented by white oval area between arrows). Arrows point to calcite (electron-dense material) on the cell surface. Cells are unstained to avoid dissolution of calcite by acidic heavy metal stains used to provide contrast to biological specimens. Scale bar equals 200 nm. (D–F) Series of transmission electron micrographs showing progression of gypsum precipitation on cell wall of *Synechococcus* (whole mounts): (D) Initiation of numerous nucleation sites on cell surface. (E) Gypsum precipitation spreading away from cell. Gypsum still appears to be covered by thin layer of bacterial slime. (F) Dividing *Synechococcus* cell shedding some of the precipitated gypsum. Scale bars equal 500 nm. (Courtesy of J. B. Thompson from Thompson and Ferris, 1990, with permission.)

(Brunskill and Ludlam, 1969). Its water is naturally alkaline (pH around 7.95) and contains on the order of 11 mM Ca^{2+}, 2.8 mM Mg^{2+}, and 10 mM SO_4^{2-}. It has an ionic strength of around 54.1 and an alkalinity of around 3.24 (Thompson and Ferris, 1990). Gypsum crystals developed on the surface of *Synechococcus* cells before calcite crystals, but the calcite deposit became more massive and less prone to being shed by the cells on division than the gypsum deposit. Calcite deposition coincided with a rise in pH in the immediate surround of the cells that was related to their photosynthetic activity. Gypsum deposition occurred in the dark as well as in light and hence was not driven by photosynthesis, as was calcite deposition. Indeed, Thompson and Ferris suggest that calcite may replace gypsum in the developing bioherm (a microbialite) in the lake. Calcite is deposited on the *Synechococcus* cells as a result of interaction between calcium ions bound at the cell surface and carbonate ions generated as a result of the photosynthetic activity of the cells. The cell-bound calcium ions also capture sulfate ions to form gypsum. This activity explains the origin of the marl and the calcified bioherm that are found in Green Lake (Thompson et al., 1990).

Instances have been found in which the calcium carbonate that formed in the lithification of some cyanobacterial mats did not originate during photosynthesis of the cyanobacteria. It originated with the activity of bacteria associated with the cyanobacteria (Chafetz and Buczynski, 1992). According to Chafetz and Buczynski, cyanobacterial stromatolites may thus owe their existence to bacterial $CaCO_3$ precipitation rather than cyanobacterial photosynthesis.

A Possible Model for Oolite Formation

A process that may serve as a model for oolite formation is that involving the deposition of carbonate on the cell surface of a marine pseudomonad, strain MB-1. Living or dead cells of this bacterium adsorb calcium and magnesium ions on its cell surface (cell wall–membrane complex) (Greenfield, 1963) which can then react with carbonate ions in solution. This process of $CaCO_3$ deposition is therefore not directly dependent on the living state of the organism. The dead cells adsorb calcium ions more extensively than they adsorb magnesium ions. Carbonate in the medium derives mostly from respiratory CO_2 produced by living cells of the organism. The conversion of CO_2 to carbonate is brought about by hydrolysis of ammonia produced from organic nitrogen compounds by actively metabolizing cells. It results in the formation of aragonite ($CaCO_3$) on the cells. These cells then serve as nuclei for further calcium carbonate precipitation (Fig. 8.6). Observations by Buczynski and Chafetz (1991) strongly support this model. A similar phenomenon was also shown with a marine yeast (Buck and Greenfield, 1964, as cited by McCallum and Guhathakurta, 1970).

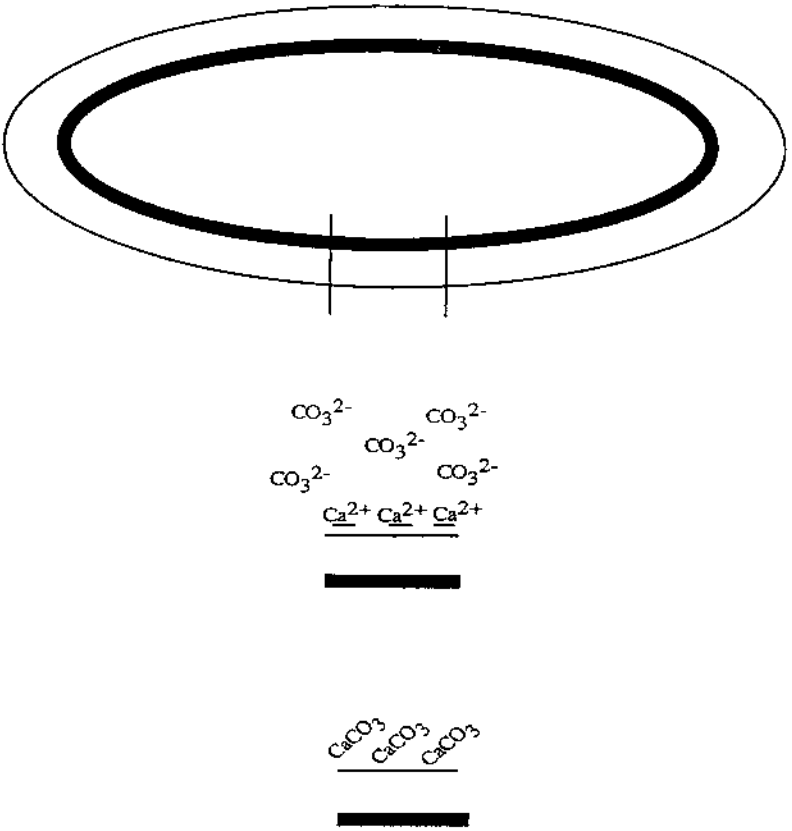
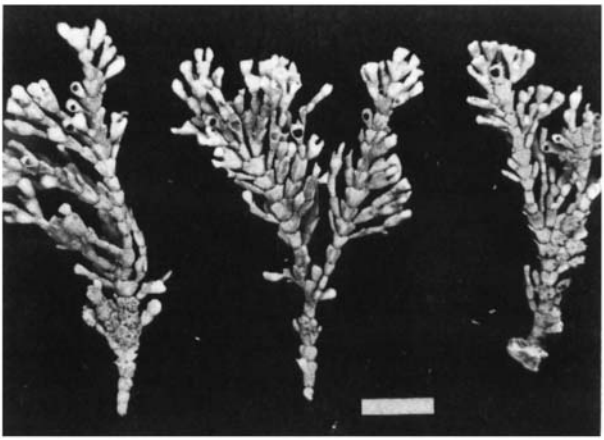


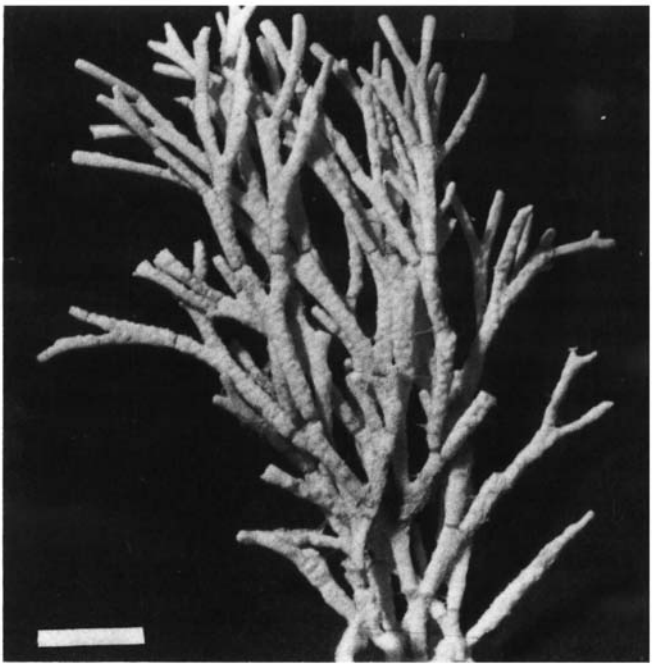
FIG. 8.6 Schematic diagram of calcium carbonate deposition on cell surface of a prokaryote (domain Bacteria), resulting in calcite or aragonite formation. See text for details.

Structural or Intracellular Carbonate Deposition by Microbes

In general, morphological and physiological studies have shown that bacteria, including cyanobacteria, and some algae cause precipitation of $CaCO_3$ mostly in the bulk phase close to the surface of their cells or at their cell surface. In contrast, some other algae and protozoa form $CaCO_3$ intracellularly and then export it to the cell surface to become support structures. Examples of eukaryotic algae that form $CaCO_3$ external to their cells include the green algae (Chlorophyceae) such as *Chara* and *Halimeda* (de Vrind-de Jong and de Vrind, 1997) and probably *Acetabularia*, *Nitella*, *Penicillus*, and *Padina* and coralline algae such as



(a)



(b)

FIG. 8.7 Articulated coralline (calcareous) algae. (a) *Arthrocardia silvae*, Johansen, California. Scale mark = 1.5 cm. (b) *Amphiroa beauvoisii*, Lamouroux, Gulf of California. Scale mark = 1.5 cm. (Courtesy of H. William Johansen.)

Arthrocardia silvae and *Amphiroa beauvoisii* (Fig. 8.7). Organisms that form $CaCO_3$ intracellularly and then export it to the cell surface include the coccolithophores (green algae) (Fig. 8.8) such as *Scyphosphaera*, *Rhabdosphaera*, and *Calciococcus* and foraminifera (Protozoa, Sarcodinae) such as *Heterostegina* and *Globigerina* (Fig. 8.9). The mineral form of the calcium carbonate that is deposited is either calcite or aragonite (Lewin, 1965). The amount of carbon incorporated into carbonate by algae as a result of photosynthesis may be a significant portion of the total carbon assimilated (Jensen et al., 1985). Wefer (1980) measured in situ $CaCO_3$ production by *Halimeda incrassata*, *Penicillus capitatus* and *Padina sanctae-crucis* in Harrington Sound, Bermuda, that amounted to approximately 50, 30, and 240 $g\,m^{-2}\,yr^{-1}$, respectively.

Chara and *Halimeda* induce calcification by creating conditions of supersaturation at specific sites at the cell surface. The sites involve internodal cells in *Chara* and intercellular spaces in *Halimeda* (de Vrind-de Jong and de Vrind, 1997). Gelatinous or mucilagenous substances in association with the cell walls of these algae may be involved in the deposition and organization of the crystalline $CaCO_3$. If the CO_2 for forming the algal carbonate depends on

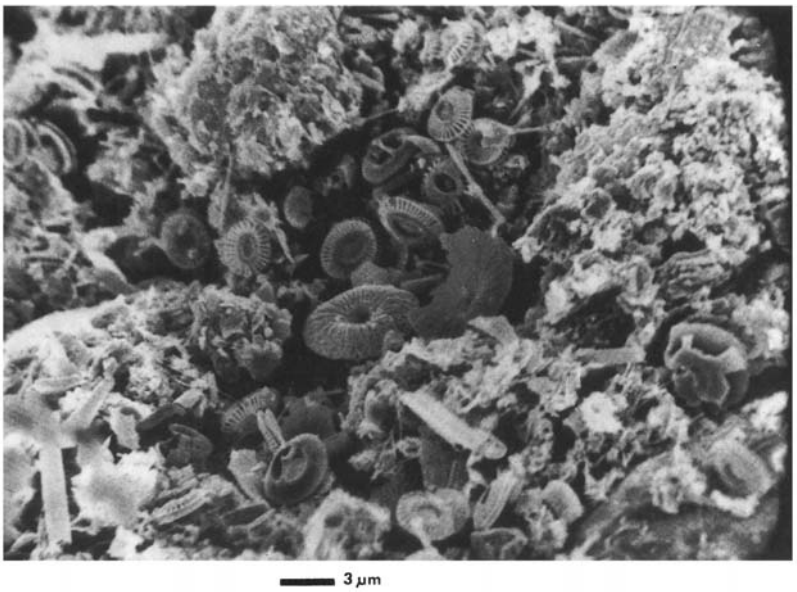
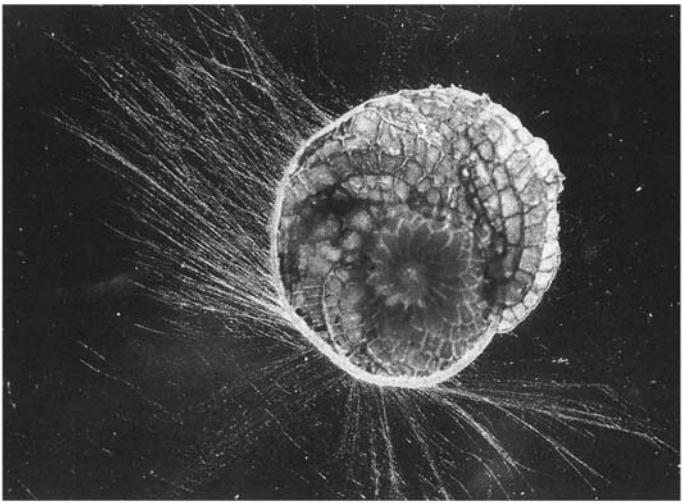
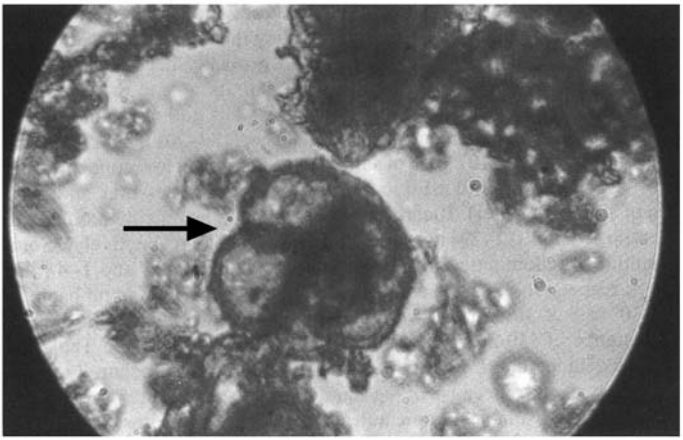


FIG. 8.8 Coccoliths, the calcareous skeletons of an alga belonging to the class Chrysophyceae (see sketch in Fig. 5.9). These specimens were found residing on the surface of a ferromanganese nodule from Blake Plateau of the Atlantic coast of the United States (From LaRock and Ehrlich, 1975. Observations of bacterial microcolonies on the surface of ferromanganese nodules from Blake Plateau by scanning electron microscopy. Microb Ecol 2:64–96. Copyright 1975 Springer-Verlag, New York, with permission.)



(a)



(b)

FIG. 8.9 Foraminifera. (a) A living foraminiferan specimen, *Heterostegina depressa*, from laboratory cultivation. Note the multichambered test and the fine protoplasmic threads projecting from the test. Test diameter, 3 mm. (Courtesy of R. Röttger. See also Röttger, 1974). (b) Foraminiferan test (arrow) in Pacific sediment: *Globigerina* (?) (×2430).

photosynthesis and came from seawater, it is likely to be enriched in ^{13}C relative to seawater CO_2, whereas if the CO_2 has a respiratory origin, the algal carbonate is likely to be enriched in ^{12}C relative to seawater (Lewin, 1965). The basis for the morphogenesis of structures as intricate as those found associated with calcareous foraminifera remains to be explained in detail. Involvement of the Golgi apparatus in the cytoplasm of the cells is likely.

Some insight into intracellular coccolith assembly has been gained. In the coccolithophore *Pleurochrysis carterae*, coccolith formation is associated with coccolithosome-containing vesicles and cisternae-containing scales in the Golgi apparatus, which is located in the cytoplasm of the cells. Once formed, the coccoliths are exported to the cell surface (van der Wal et al., 1983; de Jong et al., 1983; de Vrind-de Jong and de Vrind, 1997). By contrast, coccoliths of *Emiliania huxleyi* are formed in special vesicles called the coccolith production compartments, which may be derived by fusion of Golgi vesicles (de Vrind-de Jong and de Vrind, 1997). Acidic polysaccharides appear to play an organizing role (template?) in $CaCO_3$ deposition. In strains A92, L92, and 92D of *E. huxleyi*, acidic Ca^{2+}-binding polysaccharides have been found to inhibit precipitation of $CaCO_3$ in vitro (Borman et al., 1982,1987) and are thought to regulate $CaCO_3$ precipitation in the intracellular coccolith-forming vesicles (see de Jong et al., 1983). *Em. huxleyi* derives its carbonate mainly from photosynthesis, whereas *P. carterae* derives it from photosynthesis in the light and respiration in the dark (de Vrind-de Jong and de Vrind, 1997).

A Model for Skeletal Carbonate Formation

A possible clue to the biochemical mechanism of $CaCO_3$ deposition in cell structures, especially in protozoa (heterotrophs), was suggested in an observation of Berner (1968). He noted that during bacterial decomposition of butterfish and smelts in seawater in a sealed jar, calcium was precipitated not as $CaCO_3$ but as calcium soaps or adipocere (calcium salts of fatty acids) despite the presence of HCO_3^- and CO_3^{2-} species and an alkaline pH in the reaction mixture. The prevailing fatty acid concentration favored calcium soap formation over calcium carbonate formation. Berner suggested that in nature such soaps could later be transformed into $CaCO_3$ through mineralization of the fatty acid ligand. However, an actual analog of these reactions in calcification has not been reported to date.

McConnaughey (1991) has proposed that *Chara corallina*, a calcareous alga that lays down $CaCO_3$ pericellularly forms it at the cell surface by a process involving an ATP-driven H^+/Ca^{2+} exchange. In this process, protons produced in the reaction

$$Ca^{2+} + CO_2 + H_2O \rightarrow CaCO_3 + 2H^+ \tag{8.21}$$

which is assumed to occur on the $CaCO_3$ surface facing the cell, are exchanged for intracellular Ca^{2+}. The protons then react with HCO_3^- in the cell to generate CO_2 for photosynthesis:

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \tag{8.22}$$

In other organisms that form $CaCO_3$, calcium intended for calcium carbonate structures may first be localized by formation of an organic calcium salt at the site of calcium deposition (e.g., the plasma membrane; Golgi apparatus) (see, e.g., de Vrind-de Jong and de Vrind, 1997). It may then be converted to $CaCO_3$ in the presence of carbonic anhydrase, an enzyme that can promote the conversion of dissolved metabolic CO_2 to bicarbonate and carbonate in a reversible reaction. Carbonic anhydrase has been detected in some microalgae and cyanobacteria (Aizawa and Miyachi, 1986). Although Aizawa and Miyachi considered the activity of carbonic anhydrase from the standpoint of its role in CO_2 assimilation in photosynthesis, it may very well also play a role in CO_2 conversion to carbonate under different physiological conditions. *Hyphenomonas*, a coccolithophorid, has been reported to utilize "a hydroxyproline-proline-rich peptide and sulfated polysaccharide moieties" in $CaCO_3$ deposition (Isenberg and Lavine, 1973).

Microbial Formation of Carbonates Other Than Those of Calcium

Sodium Carbonate

Carbonate may occur in solid phases not only as calcium or calcium plus magnesium salts but also as a sodium salt (natron, $Na_2CO_3 \cdot 10H_2O$). In at least one instance, the Wadi Natrun in the Libyan desert, such a deposit has been clearly associated with the activity of sulfate-reducing bacteria (Abd-el-Malek and Rizk, 1963b). As the authors described it, the wadi (the channel of a watercourse that is dried up except during periods of rainfall; an arroyo) in this case contains a chain of small lakes 23 m below sea level. The smallest of the lakes dries up almost completely during the summer, and the larger ones dry out partially at that time. Natron is in solution in the water of all the lakes and in solid form on the bottom of some of the lakes. The water feeding the lakes is supplied partly by springs and partly by streamlets, which probably derive their water from the nearby Rosetta branch of the Nile. On its way to the lakes, the surface water passes through cypress swamps. The authors found sulfate and carbonate in high concentration in the lakes (189–204 meq of carbonate per liter and 324–1107 meq of sulfate per liter) and at low concentration in the cypress swamp (0 meq to traces of carbonate per liter and 2–13 meq of sulfate per liter). Bicarbonates occurred in significant amounts in the lakes and swamps (11–294 meq and 11–16 meq per liter, respectively). Soluble sulfides predominated in the lakes and swamps (7–13

TABLE 8.2 Chemical and Bacteriological Analyses of Water and Soil Samples from Wadi Natrun

Type and source of sample	pH range	Milliequivalents[a] of: CO_3^{2-}	HCO_3^-	SO_4^{2-}	Cl^-	S^{2-}	Total soluble salts ($g\,L^{-1}$)	Organic matter (%)	Viable counts of sulfate reducers[b] ($10^6\,mL^{-1}$)
Water									
Artesian wells	7.4–7.8	0	2–5	9–13	18–30	0.2–0.5	2–3		—[d]
Burdi swamps	6.8–7.2	0–trace	11–61	2–13	1–7	1–4	1–8		1–5
Lakes	9.5–10.1	189–240	22–294	324–1107	107–210	7–13	180–239		—[d]
Soil									
Newly cultivated uplands	7.0–7.6	0	1–2	14–18	11–19[c]	—[c]	—[c]	0.2–0.5	—[d]
About 150 m from lakes	7.2–7.5	0	2–3	12–23	1–6[c]	—[c]	—[c]	0.1–5.2	5–8
Swamps	7.4–7.8	Trace	3–11	4–7	3–8[c]	—[d]	—[c]	3.4–7.8	0.8–2

[a] Milliequivalents per liter of water and per 100 g of soil.
[b] Counts per milliliter of water and per gram of soil.
[c] Not determined.
[d] Not detected.
Source: Abd-el-Malek and Rizk (1963b), with permission.

and 1–4 meq per liter, respectively). Considerable numbers of sulfate-reducing bacteria (1×10^6–8×10^6 mL^{-1}) were detected in the swamps and in the soil at a distance of 150 m from the lakes, but not elsewhere (see Table 8.2 for more detailed data). Sulfate reduction was inferred to occur chiefly in the cypress swamps because of the significant presence of sulfate reducers and the readily available organic nutrient supply at those sites. The sulfate reduction leads to the production of bicarbonate as follows:

$$SO_4^{2-} + 2(CH_2O) \rightarrow H_2S + 2CO_2 + 2OH^- \quad (8.23)$$

$$2CO_2 + 2OH^- \rightarrow 2HCO_3^- \quad (8.24)$$

Most of the soluble products of sulfate reduction, HCO_3^- and HS^-, were found to be washed into the lakes, where, upon evaporation, they became concentrated and partially precipitated as carbonate, including natron, and sulfide salts. Some of the sulfide produced in the swamps was found to combine with iron to form ferrous sulfide, which imparts a characteristically black color to the swamps. The carbonate in the lakes resulted from the loss of CO_2 from the water to the atmosphere, promoted by the warm water temperatures, especially in summer (CO_2 solubility in water decreases with increase in temperature) [Eq. (8.20)].

Manganous Carbonate

Carbonate may also combine with manganese to form rhodochrosite ($MnCO_3$) in nature. The origin of at least some of the $MnCO_3$ deposits has been ascribed to microbial activity. An example is the occurrence of rhodochrosite together with siderite ($FeCO_3$) in Punnus-Ioki Bay of Lake Punnus-Yarvi on the Karelian peninsula in Russia (Sokolova-Dubinina and Deryugina, 1967). Lake Punnus-Yarvi is 7 km long and 1.5 km wide at its broadest part. Its greatest depth is 14 m. It is only slightly stratified thermally. The oxygen concentration in its surface water was reported by the authors to range from 11.8 to 12.1 mg L^{-1} and in its bottom water from 5.7 to 6.6 mg L^{-1}. The pH of its water was given as slightly acid (pH 6.3–6.6). The Mn^{2+} concentration ranged from 0.09 mg L^{-1} in its surface water to 0.02–0.2 mg L^{-1} in its bottom water (1.4 mg L^{-1} in winter). The lake is fed by the Suantaka-Ioki and Rennel rivers and by 24 small streams that drain surrounding swampland. The lake, in turn, feeds into the Punnus-Yarvi river. It is estimated that 48% of the water in the lake is exchanged each year. The manganese and iron in the lake are derived from surface and underground drainage from Cambrian and Quaternary glacial deposits, carrying 0.2–0.8 mg of manganese per liter and 0.4–2 mg of iron per liter. The oxidized forms of manganese and iron are incorporated into silt, where they are reduced and subsequently concentrated by upward migration to the sediment/water interface and reoxidation into lake ore. This occurs mostly in Punnus-Ioki Bay, which has oxide deposits on the sediment at water depth down to 5–7 m. The oxide layer is 5–7 cm thick.

All sediment and ore samples taken from the lake (mainly Punnus-Ioki Bay) contained manganese-reducing bacteria. They were concentrated chiefly in the upper sediment layer. They included an unidentified, non-spore-forming bacillus in addition to *Bacillus circulans* and *B. polymyxa*. Limited numbers of sulfate-reducing bacteria, which the investigators (Sokolova-Dubinina and Deryugina, 1967) attributed to the lack of extensive accumulation of organic matter, were recovered from the ore. They were associated with hydrotroilite ($FeS{\cdot}nH_2O$). Carbonates of calcium and manganese at most stations in the lake and bay were reported of low occurrence (0.1%, calculated on the basis of CO_2). However, in a limited area near the center of Punnus-Ioki Bay, ore contained as much as 4.7% calcite, 5.96% siderite, and 4.99% rhodochrosite, together with 15.8% hydrogoethite and 38.9% barium psilomelanes and wads (complex oxides of manganese) (Sokolova-Dubinina and Deryugina, 1967). The relatively localized concentration of carbonates was related by the investigators to the localized availability of organic matter and its attack by heterotrophs. The organic matter was the ultimate source of CO_2-CO_3^{2-} and the cause of the essentially low E_h. It was noted that the decaying remains of the plant life on the lakeshore accumulated in sufficient quantities in the Punnus-Ioki Bay area only where extensive carbonate ores were found. The weak sulfate-reducing activity at this location may explain the low iron and manganese sulfide formation and the significant carbonate formation. In view of the much more recent discoveries of various new types of sulfate-reducing bacteria and the ability of a number of them to use ferric iron and Mn(IV) oxides in place of sulfate as terminal electron acceptor (see Chaps. 15, 16, and 18), this site should be reinvestigated using phylogenetic probes and physiological tests.

Mixed deposits including manganous and ferrous carbonate, manganous and ferrous sulfides, and manganous and calcium-iron phosphates have been found in the sediments of the Landsort Deep in the central Baltic Sea. The site is anoxic. The minerals are thought to have formed authigenically as a result of microbial mineralization of organic matter (Suess, 1979). Here metal carbonate and sulfide deposition appeared to be compatible, perhaps because iron and manganese were available in a nonlimiting supply.

Bacterial rhodochrosite (manganous carbonate) formation in the reduction of Mn(IV) oxide has been observed in pure culture under laboratory conditions using isolate GS-15, now known as *Geobacter metallireducens* (Lovley and Phillips, 1988).

Ferrous Carbonate

Siderite ($FeCO_3$) beds in the Yorkshire Lias in England are thought to have resulted from Fe_2O_3 reduction and subsequent reaction with HCO_3^-. The bicarbonate could have resulted from microbial mineralization of organic matter. At the time of this study, formation of siderite at this spot was explained

on the basis of an exclusion of sulfate by an overlying clay layer, which blocked the entry of sulfate to the site of siderite deposition. If sulfate had entered the site, it was expected that it would have been bacterially reduced to sulfide, leading to the preferential formation of iron sulfide instead of siderite (see Sellwood, 1971).

Microbial participation in siderite formation is supported by recent observations in rapidly accreting tidal marsh sediments at very shallow depths on the Norfolk coast, England. Here, extensive bacterial decay of organic matter is occurring at low interstitial sulfate and sulfide concentrations (Pye, 1984; Pye et al., 1990). Despite the low interstitial sulfate concentration, sulfate-reducing bacteria were detected. Scanning electron microscopic examination and X-ray powder diffraction analysis of siderite concretions from this site revealed that siderite formed a void-filling cement and coating around quartz grains. Traces of greigite, iron monosulfide, and calcite were also detected (Pye, 1984). Carbon isotope fractionation studies supported a microbiological role in the formation of the siderite (Pye et al., 1990). The investigators explained the simultaneous formation of siderite and ferrous sulfide as a result of a faster rate of reduction of ferric iron than of sulfate. At the time, the investigators believed that sulfate reduction and ferric iron reduction were caused by two different types of organism. Since then, it has been shown that some sulfate reducers can use ferric iron as an alternative terminal electron acceptor in place of sulfate (Coleman et al., 1993; Lovley et al., 1993).

Coleman et al. (1993) demonstrated that *Desulfovibrio* in salt marsh sediment from the Norfolk site could reduce ferric to ferrous iron with H_2 as electron donor. When neither sulfate nor H_2 was limiting as an electron donor, both sulfate and ferric iron were reduced. However, when H_2 was limiting but sulfate was not, ferric iron seemed to be the preferential electron acceptor. Interestingly, significantly more carbonate (75%) in the siderite nodules in the marsh derived from seawater than from the degradation of organic matter (25%). Thus in the case of siderite formation in the Norfolk coastal marsh, bacterial Fe(III) reduction made a more important contribution to siderite formation than microbial mineralization of organic carbon.

Siderite formation by bacteria resident on crushed bauxite was observed in a laboratory column. The columns were fed with a sucrose–mineral salts solution. The siderite was detected by X-ray diffraction analysis of ore residue in the column at the end of the experiment. Concurrent iron sulfide formation was noted in this experiment. The source of the sulfide was the reduction of sulfate in the mineral salts solution. Sulfate-reducing bacteria as well as other bacteria were detected in the column (Ehrlich and Wickert, 1997).

Strontium Carbonate

Strontium carbonate is a little more insoluble ($K_{sol} = 10^{-8.8}$) than calcium carbonate ($K_{sol} = 10^{-8.07}$), so it should not be surprising that at appropriate

conditions Sr^{2+} can be precipitated by biogenic carbonate. Three cases of microbial strontium carbonate formation have been reported so far, all under laboratory conditions. In the first instance, Roemer and Schwartz (1965) showed that sulfate-reducing bacteria could form strontianite ($SrCO_3$) from celestite ($SrSO_4$). In the second instance, Anderson and Appanna (1994) showed that a soil strain of *Pseudomonas fluorescens* could form crystalline strontium carbonate from 5 mM strontium citrate. The strontium carbonate was identified by X-ray fluorescence spectroscopy, X-ray diffraction spectrometry, Fourier transform infrared spectroscopy, and acid treatment. The source of the carbonate was the mineralization of the citrate by the bacterium. In the third instance, Roden and Ferris (2000) demonstrated the formation of strontium carbonate during amorphous hydrous ferric oxide reduction by *Shewanella putrefaciens* CN32 in defined bicarbonate-buffered, Ca-free and Ca-amended medium. The $SrCO_3$ was incorporated in the ferrous carbonate (siderite) produced in the ferric oxide reduction at initial Sr concentrations of 0.01 and 0.1 M. According to the authors, the aqueous phase at these two Sr concentrations was undersaturated with respect to $SrCO_3$ at all times. Whether any Sr was incorporated into calcite in experiments with added calcium was not reported. Similar observations of strontium immobilization during hydrous ferric oxide reduction were made with *Shewanella alga* strain BrY (Parmar et al., 2000).

In nature, Ferris et al. (1995) observed precipitation of strontium calcite on a serpentinite outcrop in a groundwater discharge zone near Rock Creek, British Columbia, Canada as a result of photosynthetic activity of epilithic cyanobacteria, including *Calothrix*, *Synechococcus*, and *Gloeocapsa*. The strontium content of the calcite was up to 1.0 wt%. The cyanobacteria served as nucleation sites in formation of the calcite. The Ca concentration in water samples from the study site was 32–36 ppm and the Sr concentration was 5.8–6.6 ppm. The pH of the water about 2 m above the outcrop was 8.5 and at its base (0.2 m level) 8.8. The pH difference was attributed to cyanobacterial photosynthesis. The existence of the strontium in the calcite was explained as a homogeneous solid solution of $SrCO_3$ in the calcite. Such solid solution of $SrCO_3$ was also observed when calcite was precipitated by *Bacillus pasteurei* in a urea medium containing Ca^{2+} and small amounts of Sr^{2+} (Warren et al., 2001). The Sr^{2+} apparently substitutes for Ca^{2+} in some structural sites of the calcite.

Magnesium Carbonate

Microbial communities in which actinomycetes belonging to the genus *Streptomyces* predominated may play a role in the formation of hydromagnesite ($[Mg_5(CO_3)_4(OH)_2 \cdot 4H_2O]$) (Cañaveras et al., 1999). The organisms were found associated with moonmilk deposits containing hydromagnesite and needle-fiber aragonite in Altamira Cave in northern Spain.

8.3 BIODEGRADATION OF CARBONATES

Carbonates in nature may be readily degraded as a direct or indirect result of biological activity, especially microbiological activity (Golubic and Schneider, 1979). A chemical basis for this decomposition is the instability of carbonates in acid solution. For instance,

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3^- \tag{8.25}$$

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \tag{8.26}$$

$$H_2CO_3 \rightarrow H_2O + CO_2 \tag{8.27}$$

Because $Ca(HCO_3)_2$ is very soluble compared to $CaCO_3$, the $CaCO_3$ begins to dissolve even in weak acid solutions. In stronger acid solutions, $CaCO_3$ dissolves more rapidly because, as Eq. (8.27) shows, the CO_2 is likely to be lost from solution due to degassing. Therefore, from a biochemical standpoint, any microorganism that generates acidic metabolic wastes is capable of dissolving insoluble carbonates. Even the mere metabolic generation of CO_2 during respiration can have this effect, because

$$CO_2 + H_2O \rightarrow H_2CO_3 \tag{8.28}$$

and

$$H_2CO_3 + CaCO_3 \rightarrow Ca^{2+} + 2HCO_3^- \tag{8.29}$$

Thus, it is not surprising that various kinds of CO_2- and acid-producing microbes have been implicated in the breakdown of insoluble carbonates in nature.

Biodegradation of Limestone

Breakdown of lime in the cement of reservoir walls and docksides was attributed in part to bacterial action as long ago as 1899 (Stutzer and Hartleb, 1899). However, extensive investigation into microbial decay of limestone was first undertaken three decades later by Paine et al. (1933). These workers showed that both sound and decaying limestones usually carried a sizeable bacterial flora, the numbers ranging from 0 to over 8×10^6 g^{-1}. The size of the population in a particular sample seemed to depend in part on the environment around the stone. As one might expect, the surface of the limestone was generally more densely populated than its interior. The authors suggested that in many limestones the bacteria were unevenly distributed, inhabiting pockets and interstices in the limestone structures. The kinds of bacteria found in limestones that they examined included gram-variable and gram-negative rods and cocci. Spore formers, such as *Bacillus mycoides*, *B. megaterium*, and *B. mesentericus*, appeared to have been rare or absent. The investigators performed an experiment to estimate the rate of limestone decay through bacterial action under controlled

conditions. They employed a special apparatus in which evolved CO_2 was trapped in barium hydroxide solution. They found 0.18 mg of CO_2 per hour per 350 g of stone to be evolved in one case, and 59 mg of CO_2 per hour per 350 g of stone in another. In the latter case they calculated from the data that 28 g of CO_2 would have been evolved from 1 kg of stone in 1 year. As expected, the rate of CO_2 evolution from decaying stone was greater than from sound stone. Although in these experiments organic acids and CO_2 from heterotrophic metabolism of organic matter were the cause of the observed limestone decay, autotrophic nitrifying and sulfur-oxidizing bacteria were also shown to be able to promote limestone decay. These organisms accomplished it through the production of nitric and sulfuric acids from ammonia and reduced sulfur, respectively. Nitrifying and sulfur-oxidizing bacteria were detected by Paine et al. (1933) in some limestone samples.

In a much later study, variable numbers of fungi, algae, and ammonifying, nitrifying, and sulfur-oxidizing bacteria were found on the surface of some limestones in Germany (Krumbein, 1968). Krumbein found that the number of detectable organisms depended on the type of stone, the length of elapsed time since the collection of the stone from a natural site, the surface structure of the stone (i.e., the degree of weathering), the cleanliness of the stone, and the climatic and microclimatic conditions prevailing at the collection site. In the case of strongly weathered stone, the bacteria had sometimes penetrated the stone to a depth of 10 cm. Ammonifying bacteria were generally most numerous. Nitrogen-fixing bacteria were few, and denitrifiers were absent. The number of bacteria was not directly related to the presence of lichens or algae. The greatest number of ammonifiers were found on freshly collected and weathered stone. This was related to the pH of the stone surface (pH 8.1–8.3 in water extract). Sulfur oxidizers were more numerous in city environments, where the atmosphere contains more oxidizable sulfur compounds than in the countryside. The concentration of nitrifiers on limestone could not be correlated with city and country atmospheres. The number of ammonifiers on limestone was also found to be greater in stone exposed to city air than in stone exposed to country air. This can be explained on the basis that city air contains more organic pollutants that can serve as nutrient for these bacteria than does country air. Laboratory experiments by Krumbein confirmed the weathering of limestone by its natural flora. The ammonifiers in these observations were less directly responsible for the weathering of the limestone than they were in generating ammonia from which the nitrifying bacteria could form nitric acid, which then corroded the limestone.

In yet another important study of the decomposition of limestone by microbes, numerous bacteria and fungi were isolated from a number of samples (Wagner and Schwartz, 1965). The active organisms appeared to weather the stone through production of oxalic and gluconic acids. The investigators also noted the presence of nitrifying bacteria and thiobacilli in their samples and

suggested that the corresponding mineral acids produced by these organisms also contributed to the weathering of the stone.

Marble, a metamorphic type of $CaCO_3$ rock, can also be attacked by microorganisms. Figure 8.10 shows the effect of microcolonial fungi. *Micrococcus halobius* was shown to colonize surfaces on Carrara marble slabs, forming biofilm and producing gluconic, lactic, pyruvic, and succinic acids from glucose that etched the marble surfaces (Urzì et al., 1991). This organism also caused a discoloration of the marble surface, suggesting that natural patina on marble structures may have a microbial origin.

The microbial weathering of rock surfaces such as marble, basalt, and granite may involve not only dissolution but also precipitation of new secondary minerals. The new minerals appear as surface crusts and may include calcite, apatite, and wilkeite (Urzì et al., 1999).

Black fungi of the Dematiaceae have been implicated in the destruction of some marble and limestone (Gorbushina et al., 1994). However, instead of attacking the marble through formation of corrosive metabolic products such as acids, the fungus appears to attack the marble by exerting physical pressure in pores and crevices where it grows and by changing water activity in its superficial polymers and around the cells in the stone. The melanin pigment produced by the fungus has been implicated in the blackening of surfaces of marble structures.

Cave formation in a limestone region (karst) is under the influence of various microbes. Some microbes, including autotrophs and heterotrophs, generate the corrosive agents that attack the $CaCO_3$ of limestone and dissolve it. Others may reduce oxidized sulfur minerals like gypsum or oxides of iron or manganese (see Herman, 1994 and other papers cited therein). Some caves, like Movile cave in Rumania, which receives little input from the surface environment, have developed light-independent ecosystems in which the primary producers are chemosynthetic instead of photosynthetic autotrophs (Sarbu et al., 1994,1996).

Cyanobacteria, Algae, and Fungi That Bore into Limestone

Endolithic cyanobacteria, algae, and fungi have been found to cause local dissolution of limestone, thereby forming tubular passages in which they can grow (Fig. 8.11) (Golubic et al., 1975). The kinds of limestone they attack in nature include coral reefs, beach rock, and other types. Active algae include some green, brown, and red algae (Golubic, 1969). The mechanism by which any of these organisms bore into limestone is not understood. Some filamentous boring cyanobacteria possess a terminal cell that is directly responsible for the boring action, presumably dissolution of the carbonate (Golubic, 1969). Different boring microorganisms form tunnels of characteristic morphology (Golubic et al., 1975). In a pure mineral such as Iceland spar, boring follows the planes of crystal

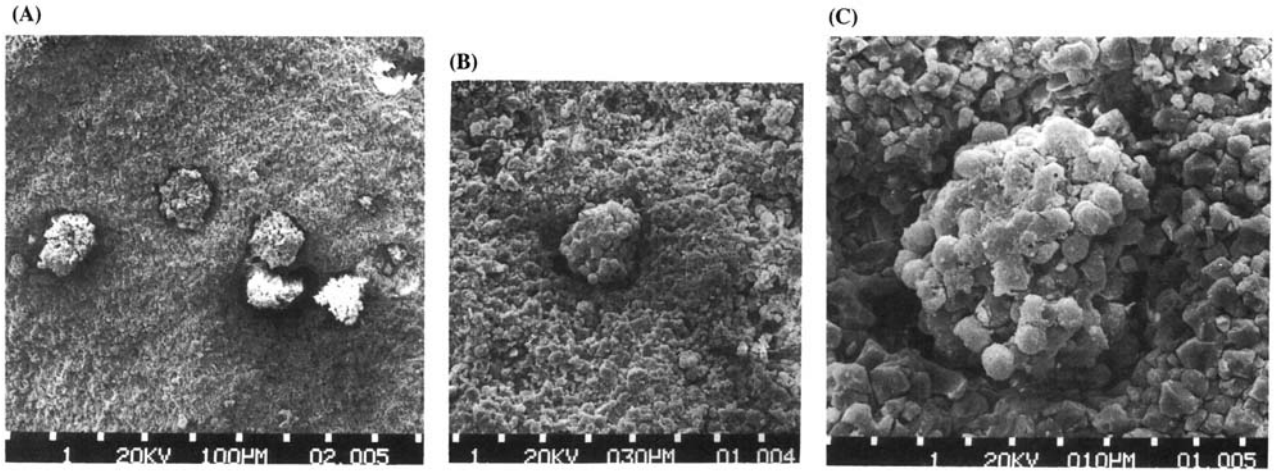
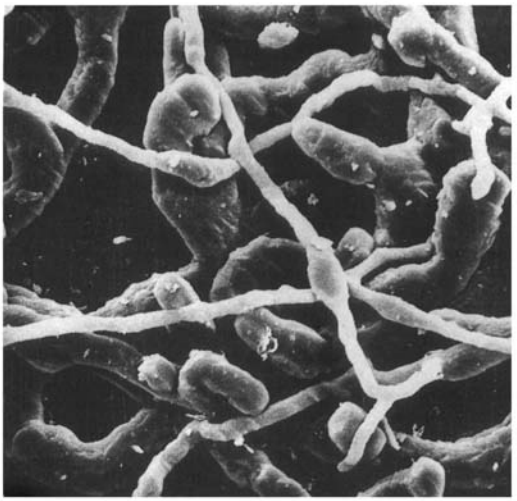


FIG. 8.10 (A–C) SEM photomicrographs showing a section of marble from the Dionysos Theater, Acropolis, Athens, Greece, at three different magnifications (note scale and scale marks on the bottom of each photograph). The marble has been extensively corroded by "biopitting." Deep holes of different sizes (between 2 μm and 5 mm in diameter and depth) were incised chemically (etched by metabolically produced substances) and physically (mechanical action) by black yeasts and meristematically growing yeastlike fungi. The microcolonial fungi can be confused with algae in SEM micrographs. The fungi have a cell size very similar to that of the marble grain. The chemical and physical corrosive action of these fungi have been demonstrated in laboratory experiments (Anagnostidis et al., 1992). (Courtesy of Wolfgang E. Krumbein.)

(a)



(b)

FIG. 8.11 Microorganisms that bore into limestone. (a) Limestone sample experimentally recolonized by the cyanobacterium *Hyella balani* Lehman (×234). The exposed tunnels are the result of boring by the cyanobacterium. (b) Casts of the borings of the green alga *Eugamantia sacculata* Kormann (larger filaments) and the fungus *Ostracoblabe implexa* Bornet and Flahault in calcite spar (×2000). The casts were made by infiltrating a sample of fixed and dehydrated bore mineral with synthetic resin and then etching sections of the embedded material (e.g., with dilute mineral acid) to expose the casts of the organism. (Courtesy of S. Golubic.)

twinning, diagonal to the main cleavage planes (Golubic et al., 1975). The depth to which cyanobacteria and algae bore into limestone is limited by light penetration in the rock, because they need light for photosynthesis. Boring cyanobacteria may have unusually high concentrations of phycocyanin, an accessory pigment of the photosynthetic apparatus, to compensate for the low light intensity in the limestone. In contrast, boring fungi are not limited by light penetration. Being incapable of photosynthesis, they have no need for light.

Endolithic fungi and cyanobacteria or algae in limestone and sandstone may form a special relationship in the form of lichens (Fig. 8.12). The cyanobacteria or algae in these associations share the carbon that they fix with the fungi, while the fungi share minerals that they mobilize and some other less well defined functions with the cyanobacteria or algae. These lichens, although growing within limestone, may serve as food to some snails, as has been reported from the Negev Desert in Israel (Shachack et al., 1987). The snails have a tonguelike organ in their mouth, the **radula**, which has toothlike structures embedded in it that are useful for abrading. The toothlike structures frequently consist of the iron mineral hematite. The snails scrape the surface of the limestone beneath which the lichens grow with their radula in order to feed on the lichens, their preferred food. They ingest some of the pulverized limestone with the lichen and leave behind a trail of this limestone powder as they move over the limestone surface. It has been estimated that in the Negev Desert this weathering affects

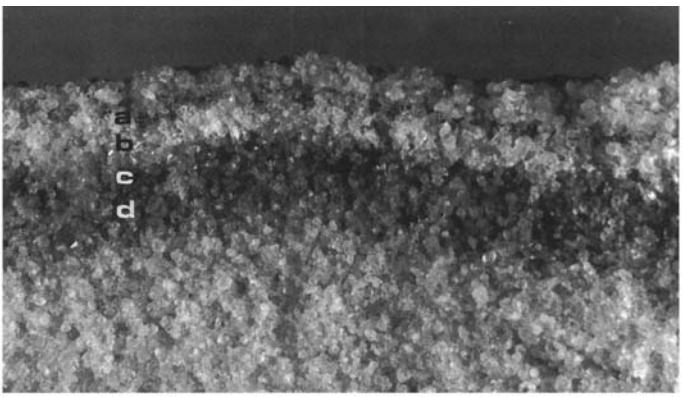


FIG. 8.12 Cryptoendolithic microorganisms in vertically fractured Beacon sandstone. (a) Lichen (small black bodies between rock particles), (b) zone of fungus filaments, (c) yellowish-green zone of unicellular cyanobacterium. (d) blue-green zone of unicellular cyanobacterium. The color difference between (c) and (d) is not apparent in this black-and-white photograph. Sample A 76-77/36, north of Mount Dido, elevation 1750 m, magnification ×4.5. (From Friedmann, EI. 1977. Microorganisms in Antartic desert rock from dry valleys and Dufek Massif. Antartic Journal of the United States, courtesy of the author.)

0.7–1.1 metric tons of rock per hectare per year (Shachak et al., 1987). A similar biological weathering phenomenon was previously noted on some reef structures in Bermuda (see Golubic and Schneider, 1979).

Cyanobacteria and fungi inhabit not only limestone but also other porous rock. To distinguish among rock-inhabiting microbes, the term **euendoliths** has been coined for true boring microbes in limestone. Opportunists that invade preexistent pores of rock where they may cause alteration of the rock in the area that they inhabit are classified as **cryptoendoliths**. Those that invade preexistent cracks and fissures without altering the rock structure are classified as **chasmoendoliths** (Golubic et al., 1981).

In some environments, such as Antarctic dry valleys, cryptoendolithic cyanobacteria (Friedmann and Ocampo, 1976) and lichens (in this instance a symbiotic association of a green alga and a filamentous fungus) (Friedmann, 1982; Friedmann and Ocampo-Friedmann, 1984) inhabit superficial cavities in sandstone, 1–2 mm below the surface (Fig. 8.12). The near-surface locale in the sandstone (orthoquartzite) is the major habitat for microorganisms in this inhospitable environment (Friedmann, 1982). These cryptoendolithic microorganisms appear to differ from the boring microbes in that they are not directly responsible for cavity formation in the sandstone but instead invade preexistent pores. However, they promote exfoliation of the sandstone surface, a physical process facilitated through solubilization of the cementing substance ($CaCO_3$?) that holds the quartz grains together (Friedmann and Weed, 1987). The lichen activity may manifest itself visibly by mobilization of iron in the region of lichen activity. Exfoliation may expose the lichens to the external environment from which, if they survive the exposure, they may reinvade the sandstone through pores. Fissures in granite and grandiorite in the Antarctic dry valleys may also be inhabited by lichens and coccoid cyanobacteria (Friedmann, 1982). These organisms are clearly not boring microbes.

Cryptoendoliths are not unique to the Antarctic dry valleys but are also found in hot desert environments (e.g., Mojave Desert, California; Sonoran Desert, Mexico; Negev, Israel) (Friedmann, 1980). Because the hot deserts, like the cold Antarctic deserts, represent extreme environments in which moisture is a limiting factor to survival, rock interiors afford protection and permit life to persist.

8.4 BIOLOGICAL CARBONATE FORMATION AND DEGRADATION AND THE CARBON CYCLE

The biological aspect of the carbon cycle involves chiefly the fixation of inorganic carbon (CO_2 or its equivalent) as organic carbon and the remineraliza-

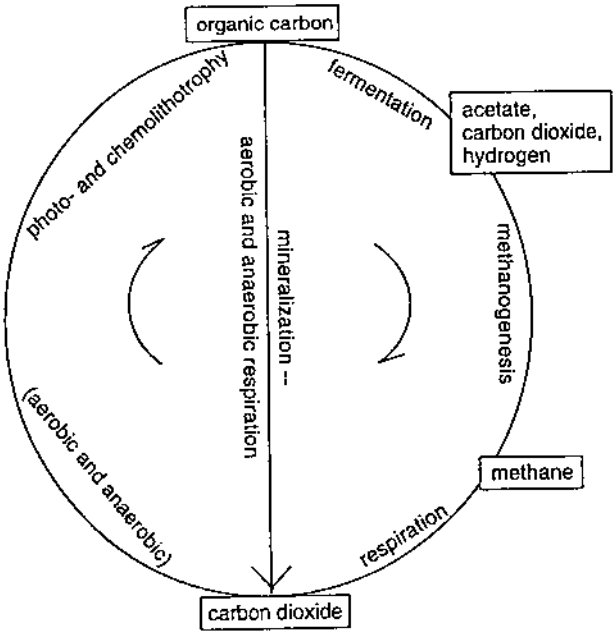


FIG. 8.13 The carbon cycle.

tion of some of this organic carbon to inorganic carbon (Fig. 8.13). The carbon cycle operates in such a way that a certain portion of the biologically fixed carbon is transitionally immobilized as **standing crop** (e.g., Smith, 1981). However, the standing crop undergoes continual turnover at such a rate that its size does not markedly change unless it is subjected to some major environmental change.

Some sediments may become a permanent sink of organic matter trapped in it. Through burial, such organic matter is removed from the carbon cycle and may be gradually transformed into coal, petroleum, bitumen, kerogen, and/or natural gas (see Chap. 21) or, very rarely, graphite or diamonds. The carbon in these substances reenters the carbon cycle with few exceptions only through human intervention (the exploitation of fossil fuels).

As this chapter has demonstrated, inorganic carbon may also be removed from the carbon cycle through biotransformation into inorganic carbonates. Such carbon when trapped in sediments may not reenter the active carbon cycle for extended geologic time intervals. Reentry of carbon into the cycle, as this chapter has shown, is under extensive biological control. Indeed, the biological component of the carbon cycle as a whole exerts the chief directing influence over it.

For more detailed discussion of the carbon cycle on Earth, the following references should be examined: Bolin (1970), Golubic et al. (1979), Krumbein and Swart (1983), and Post et al. (1990).

8.5 SUMMARY

Carbon dioxide is trapped on Earth mainly as calcium and calcium-magnesium carbonates but also to a much lesser extent as carbonates of iron, manganese, sodium, magnesium, and strontium. In many cases, these carbonates are of biogenic origin. Calcium or calcium-magnesium carbonates may be precipitated by bacteria, cyanobacteria, and fungi by (1) aerobic or anaerobic oxidation of carbon compounds devoid of nitrogen in neutral or alkaline environments with a supply of calcium and magnesium counter ions, (2) aerobic or anaerobic oxidation of organic nitrogen compounds in unbuffered environments with a supply of calcium and magnesium counter ions, (3) $CaSO_4$ reduction by sulfate-reducing bacteria, (4) the hydrolysis of urea in the presence of Ca and Mg counter ions, or (5) photo- and chemosynthetic autotrophy in the presence of Ca and Mg counter ions.

The complete mechanism of calcium carbonate precipitation by living organisms is not understood in many cases, although it always depends on metabolic CO_2 production or CO_2 consumption and the relative insolubility of calcium carbonate. Bacteria and fungi deposit it extracellularly. Bacterial cells may also serve as nuclei around which calcium carbonate is laid down. Some algae and protozoa form structural carbonate either extracellularly or intracellularly. Structural $CaCO_3$ formation may involve local fixation of calcium prior to its reaction with carbonate, regardless of whether the carbonate is of photosynthetic or respiratory origin.

Microbial calcium carbonate precipitation has been observed in soil and in freshwater and marine environments. Sodium carbonate (natron) deposition associated with microbial sulfate-reducing activity has been noted in the hot climate of the Wadi Natrun in the Libyan Desert. Ferrous carbonate deposition associated with microbial activity has been noted in some special coastal marine environments. Manganese carbonate deposition associated with microbial activity has been noted in a freshwater lake. Both ferrous and manganous carbonate formation have been observed in the laboratory with pure bacterial cultures.

Insoluble carbonates may be broken down by microbial attack. This is usually the result of organic and inorganic acid formation by the microbes but may also involve physical processes. A variety of bacteria, fungi, and even algae have been implicated. Such activity is evident on limestones and marble used in building construction, but it is also evident in natural limestone formations such as coral reefs, where limestone-boring algae and fungi are active in the break-

down process. Bacteria and fungi contribute to the discoloration of structural limestone and marble and may be the cause of patina.

REFERENCES

Abd-el-Malek Y, Rizk SG. 1963a. Bacterial sulfate reduction and the development of alkalinity. II. Laboratory experiments with soils. J Appl Bacteriol 26:14–19.

Abd-el-Malek Y, Rizk SG. 1963b. Bacterial sulfate reduction and the development of alkalinity. III. Experiments under natural conditions in the Wadi Natrun. J Appl Bacteriol 26:20–26.

Aizawa K, Miyachi S. 1986. Carbonic anhydrase and CO_2 concentration mechanism in microalgae and cyanobacteria. FEMS Microbiol Rev 39:215–233.

Anagnostidis K, Gehrmann K, Gross M, Krumbein WE, Lisi S, Panazidou A, Urzì C, Zagari M. 1992. Biodeterioration of marbles of the Parthenon and Propylea, Acropolis, Athens—Associated organisms, decay, and treatment suggestions. In: Decrouez D, Chamay J, Zezza F, eds. II International Symposium for the Conservation of Monuments in the Mediterranean Basin. Genève, Switzerland: Musée d'Histoire Naturelle, pp 305–325.

Anderson S, Appanna VD. 1994. Microbial formation of crystalline strontium carbonate. FEMS Microbiol Lett 116:43–48.

Ashirov KB, Sazonova IV. 1962. Biogenic sealing of oil deposits in carbonate reservoirs. Mikrobiologiya 31:680–683 (Engl transl pp 555–557).

Bavendamm W. 1932. Die mikrobiologische Kalkfällung in der tropischen See. Arch Mikrobiol 3:205–276.

Berg GW. 1986. Evidence for carbonate in the mantle. Nature (Lond) 324:50–51.

Berner RA. 1968. Calcium carbonate concretions formed by the decomposition of organic matter. Science 159:195–197.

Bolin B. 1970. The carbon cycle. Sci Am 223:124–132.

Bonatti E. 1966. Deep-sea authigenic calcite and dolomite. Science 152:534–537.

Borman AH, de Jong EW, Huizinga M, Kok DJ, Bosch L. 1982. The role of $CaCO_3$ crystallization of an acid Ca^{2+}-binding polysaccharide associated with coccoliths of *Emiliania huxleyi*. Eur J Biochem 129:179–183.

Borman AH, de Jong EW, Thierry R, Westbroek P, Bosch L. 1987. Coccolith-associated polysaccharides from cells of *Emiliania huxleyi* (Haptophyceae). J Phycol 23:118–123.

Bowen HJM. 1979. *Environmental Chemistry of the Elements*. London: Academic Press.

Brunskill GJ, Ludlam SD. 1969. Fayetteville Green Lake, New York. I. Physical and chemical limnology. Limnol Oceanogr 14:817–829.

Buchanan RE, Gibbons NE, eds. 1974. *Bergey's Manual of Determinative Bacteriology.* 8th ed. Baltimore, MD: Williams and Wilkins.

Buck JD, Greenfield LJ. 1964. Calcification in marine-occurring yeast. Bull Mar Sci Gulf Carib 14:239–245.

Buczynski C, Chafetz HS. 1991. Habit of bacterially induced precipitates of calcium carbonate and the influence of medium viscosity on mineralogy. J Sedim Petrol 61:226–233.

Cañaveras JC, Hoyos M, Sanchez-Moral S, Sanz-Rubio E, Bedoya J, Soler V, Groth I, Schumann P, Laiz L, Gonzalez I, Saiz-Jimenez C. 1999. Microbial communities associated with hydromagnesite and needle-fiber aragonite deposits in a karstic cave (Altamira, northern Spain). Geomicrobiol J 16:9–25.

Chafetz HS, Buczynzki C. 1992. Bacterially induced lithification of microbial mats. Palaios 7:277–293.

Coleman ML, Hedrick DF, Lovley DR, White DC, Pye K. 1993. Reduction of Fe(III) in sediments by sulfate-reducing bacteria. Nature (Lond) 361:436–438.

De Boer W, la Rivière J, Schmidt K. 1971. Some properties of *Achromobacter oxaliferum*. Leeuwenhoek 37:553–556.

de Jong, EW, van der Wal P, Borman AH, de Vrind JPM, Van Emburg P, Westbroek P, Bosch L. 1983. Calcification in coccolithophorids. In: Westbroek P, de Jong WE, eds. Biomineralization and Biological Metal Accumulation. Dordrecht, Holland: Reidel, pp 291–301.

de Vrind-de Jong EW, de Vrind JPM. 1997. Algal deposition of carbonates and silicates. In: Banfield JG, Nealson KH, eds. Geomicrobiobiology: Interactions Between Microbes and Minerals. Rev Mineral Vol 35. Washington, DC: Mineral Soc Am, pp 267–307.

del Moral A, Roldan E, Navarro J, Monteoliva-Sanchez M, Ramos-Cormenzana A. 1987. Formation of calcium carbonate crystals by moderately halophilic bacteria. Geomicrobiol J 5:79–87.

Drew GH. 1911. The action of some denitrifying bacteria in tropical and temperate seas, and the bacterial precipitation of calcium carbonate in the sea. J Mar Biol Assoc UK 9:142–155.

Drew GH. 1914. On the precipitation of calcium carbonate in the sea by marine bacteria, and on the action of denitrifying bacteria in tropical and temperate seas. Carnegie Inst Washington, Publ 182, 5:7–45.

Ehrlich HL, Wickert LM. 1997. Bacterial action on bauxites in columns fed with full-strength and dilute sucrose–mineral salts medium. In: Lortie L, Bédard P, Gould WD, eds. Biotechnology and the Mining Environment. Proc 13th Annu Gen Meet Biominet Sp 97-1. Ottawa, Canada: CANMET, Nat Resources Canada.

Feeley HW, Kulp JL. 1957. Origin of Gulf Coast salt-dome sulfur deposits. Bull Am Assoc Petrol Geol 41:1802–1853.

Fenchel T, Blackburn TH. 1979. Bacteria and Mineral Cycling. London: Academic Press.

Ferrer MR, Quevedo-Sarmiento J, Bejar V, Delgado R, Ramos-Cormenzana A, Rivadeneyra MA. 1988a. Calcium carbonate formation by *Deleya halophila*: Effect of salt concentration and incubation temperature. Geomicrobiol J 6:49–57.

Ferrer MR, Quevedo-Sarmiento J, Rivadeneyra MA, Bejar V, Delgado R, Ramos-Cormenzana A. 1988b. Calcium carbonate precipitation by two groups of moderately halophilic microorganisms at different temperatures and salt concentrations. Curr Microbiol 17:221–227.

Ferris FG, Fratton CM, Gerits JP, Schultze-Lam S, Lollar BS. 1995. Microbial precipitation of a strontium calcite phase at a groundwater discharge zone near Rock Creek, British Columbia, Canada. Geomicrobiol J 13:57–67.

Friedmann EI. 1977. Microorganisms in Antarctic desert rocks from dry valleys and Dufek Massif. Antarctic J US 12:26–30.

Friedmann EI. 1980. Endolithic microbial life in hot and Cold Deserts. Orig Life 10:223–235.

Friedmann EI. 1982. Endolithic microorganisms in the Antarctic cold desert. Science 215:1045–1053.

Friedmann EI, Ocampo R. 1976. Endolithic blue-green algae in the Dry Valleys: Producers in the Antarctic desert ecosystem. Science 193:1247–1249.

Friedmann EI, Weed R. 1987. Microbial trace-fossil formation, biogenous and abiotic weathering in the Antarctic Cold Desert. Science 236:703–705.

Friedmann EI, Ocampo-Friedmann R. 1984. Endolithic microorganisms in extreme dry environments: Analysis of a lithobiontic microbial habitat. In: Klug MJ, Reddy CA, eds. Current Perspectives in Microbial Ecology. Proc Third Int Symp Microb Ecol. Washington, DC: Am Soc Microbiol pp 177–185.

Friedmann EI, Roth WC, Turner JB, McEwan RS. 1972. Calcium oxalate crystals in the aragonite-producing green alga *Penicillus* and related genera. Science 177:891–893.

Fujita Y, Ferris FG, Lawson RD, Colwell FS, Smith RW. 2000. Calcium carbonate precipitation by ureolytic subsurface bacteria. Geomicrobiol J 17:305–318.

Golubic S. 1969. Distribution, taxonomy, and boring patterns of marine endolithic algae. Am Zool 9:747–751.

Golubic S. 1973. The relationship between blue-green algae and carbonate deposits. In: Carr NG, Whitton BA, eds. The Biology of Blue Green Algae. Oxford, UK: Blackwell Sci, pp 434–472.

Golubic S, Schneider J. 1979. Carbonate dissolution. In: Trudinger PA, Swaine DJ, eds. Biogeochemical Cycling of Mineral-Forming Elements. Amsterdam: Elsevier, pp 107–129.

Golubic S, Perkins RD, Lukas KJ. 1975. Boring microorganisms and microborings in carbonate substrates. In: Frey RW, ed. The Study of True Fossils. New York: Springer-Verlag, pp 229–259.

Golubic S, Krumbein WE, Schneider J. 1979. The carbon cycle. In: Trudinger PA, Swaine DJ, eds. Biogeochemical Cycling of Mineral Forming Elements. Amsterdam: Elsevier, pp 29–45.

Golubic S, Friedmann I, Schneider J. 1981. The lithobiontic ecological niche, with special reference to microorganisms. J Sediment Petrol 51:475–478.

Gorbushina AA, Krumbein WE, Hamman CH, Panina L, Soukharjevski S, Wollenzien U. 1994. Role of black fungi in color change and biodeterioration of antique marbles. Geomicrobiol J 11:205–211.

Gorlenko VM, Chebotarev EN, Kachalkin VI. 1974. Microbial oxidation of hydrogen sulfide in Lake Veisovo (Slavyansk Lake). Mikrobiologiya 43:530–534 (Engl transl pp 450–453).

Greenfield LJ. 1963. Metabolism and concentration of calcium and magnesium and precipitation of calcium carbonate by a marine bacterium. Ann NY Acad Sci 109:23–45.

Herman JS. 1994. Karst geomicrobiology and redox geochemistry: State of the science. Geomicrobiol J 12:137–140.

Isenberg HD, Lavine LS. 1973. Protozoan calcification. In: Zipkin I, ed. Biological Mineralization. New York: Wiley, pp 649–686.

Jensen PR, Gibson RA, Littler MM, Littler DS. 1985. Photosynthesis and calcification in four deep-water *Halimeda* species (Chlorophyceae, Caulerpales). Deep-Sea Res 32:451–464.

Krumbein WE. 1968. Zur Frage der biologischen Verwitterung: Einfluss der Mikroflora auf die Bausteinverwitterung und ihre Abhängigkeit von adaphischen Faktoren. Z Allg Mikrobiol 8:107–117.

Krumbein WE. 1974. On the precipitation of aragonite on the surface of marine bacteria. Naturwissenschaften 61:167.

Krumbein WE. 1979. Photolithotrophic and chemoorganotrophic activity of bacteria and algae as related to beachrock formation and degradation (Gulf of Aqaba, Sinai). Geomicrobiol J 1:139–203.

Krumbein WE, Giele C. 1979. Calcification in a coccoid cyanobacterium associated with the formation of desert stromatolites. Sedimentology 26:593–604.

Krumbein WE, Potts M. 1979. *Girvanella*-like structures formed by *Plectonema gloeophilum* (Cyanophyta) from the Borrego Desert in southern California. Geomicrobiol J 1:211–217.

Krumbein WE, Swart PK. 1983. The microbial carbon cycle. In: Krumbein WE, ed. Microbial Geochemistry. Oxford, UK: Blackwell, pp 5–62.

LaRock PA, Ehrlich HL. 1975. Observations of bacterial microcolonies on the surface of ferromanganese nodules from Blake Plateau by scanning electron microscopy. Microb Ecol 2:84–96.

Latimer WM, Hildebrand JH. 1942. Reference Book of Inorganic Chemistry. Rev ed. New York: Macmillan.

Lewin J. 1965. Calcification. In: Lewin RA, ed. Physiology and Biochemistry of Algae. New York: Academic Press, pp 457–465.

Lipman CB. 1929. Further studies on marine bacteria with special reference to the Drew hypothesis on $CaCO_3$ precipitation in the sea. Publ 391. Washington, DC: Carnegie Inst, 26:231–248.

Lovley DR, Phillips EJP. 1988. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron and manganese. Appl Environ Microbiol 54:1472–1480.

Lovley DR, Roden EE, Phillips EJP, Woodward JC. 1993. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Marine Geol 113:41–53.

Lowenstam HA, Epstein S. 1957. On the origin of sedimentary aragonite needles of the Great Bahama Bank. J Geol 65:364–375.

Marine Chemistry. 1971. A report of the Marine Chemistry Panel of the Committee of Oceanography. Nat Acad Sci, Washington, DC.

McCallum MF, Guhathakurta K. 1970. The precipitation of calcium carbonate from seawater by bacteria isolated from Bahama Bank sediments. J Appl Bacteriol 33:649–655.

McConnaughey T. 1991. Calcification in *Chara corallina*: CO_2 hydroxylation generates protons for biocarbonate assimilation. Limnol Oceanogr 36:619–628.

Monty CLV. 1967. Distribution and structure of recent stromatolitic algal mats, Eastern Andros Island, Bahamas. Ann Soc Geol Belgi 90:55–99.

Monty CLV. 1972. Recent algal stromatolitic deposits, Andros Island, Bahamas. Preliminary Report. Geol Rundschau 61:742–783.

Morita RY. 1980. Calcite precipitation by marine bacteria. Geomicrobiol J 2:63–82.
Moore JN. 1983. The origin of calcium carbonate nodules forming on Flathead Lake delta, northwestern Montana. Limnol Oceanogr 28:646–654.
Murray J, Irvine R. 1889/1890. On coral reefs and other carbonate of lime formations in modern seas. Proc Roy Soc Lond A 17:79–109.
Nadson GA. 1903. Die Mikroorganismen als geologische Faktoren: I. über die Schwefelwasserstoffgährung und über die Beteiligung der Mikrorganismen bei der Bildung des schwazen Schlammes (Heilschlammes). Arb d Comm Erf D Mineralseen bei Slawansk, St. Petersburg.
Nadson GA. 1928. Beitrag zur Kenntniss der bakteriogenen Kalkablagerung. Arch Hydrobiol 191:154–164.
Nazina TN, Rozanova EP, Kuznetsov SI. 1985. Microbial oil transformation processes accompanied by methane and hydrogen sulfide formation. Geomicrobiol J 4:103–130.
Nekrasova VK, Gerasimenko LM, Romanova AK. 1984. Precipitation of calcium carbonate in the presence of cyanobacteria. Mikrobiologiya 53:833–836 (Engl transl pp 691–694).
Novitsky JA. 1981. Calcium carbonate precipitation by marine bacteria. Geomicrobiol J 2:375–388.
Paine SG, Lingood FV, Schimmer F, Thrupp TC. 1933. IV. The relationship of microorganisms to the decay of stone. Roy Soc Phil Trans 222B:97–127.
Parmar N, Warren LA, Roden EE, Ferris FG. 2000. Solid phase capture of strontium by the iron reducing bacteria *Shewanella alga* strain BrY. Chem Geol 169:281–288.
Pentecost A. 1978. Blue-green algae and freshwater carbonate deposits. Proc Roy Soc Lond B 200:43–61.
Pentecost A. 1988. Growth and calcification of the cyanobacterium *Homoeothrix crustacea*. J Gen Microbiol 134:2665–2671.
Pentecost A. 1995. Significance of the biomineralizing microniche in a *Lyngbya* (Cyanobacterium) travertine. Geomicrobiol J 13:213–222.
Pentecost A, Bauld J. 1988. Nucleation of calcite on the sheaths of cyanobacteria using a simple diffusion cell. Geomicrobiol J 6:129–135.
Pentecost A, Spiro B. 1990. Stable carbon and oxygen isotope composition of calcites associated with modern freshwater cyanobacteria and algae. Geomicrobiol J 8:17–26.
Post WH, Peng T-H, Emanuael WE, King AW, Dale H, DeAngelis DL. 1990. The global carbon cycle. Am Sci 78:310–326.
Pye K. 1984. SEM analysis of siderite cements in intertidal marsh sediments, Norfolk, England. Mar Geol 56:1–12.
Pye K, Dickson AD, Schiavon N, Coleman ML, Cox M. 1990. Formation of siderite-Mg-calcite-iron sulfide concentrations in intertidal marsh and sandflat sediments, north Norfolk, England. Sedimentology 37:325–343.
Rivadeneyra MA, Perez-Garcia I, Salmeron V, Ramos-Cormenazana A. 1985. Bacterial precipitation of calcium carbonate in presence of phosphate. Soil Biol Biochem 17:171–172.
Rivadeneyra MA, Delgado R, Quesada E, Ramos-Cormenzana A. 1991. Precipitation of calcium carbonate by *Deleya halophila* in media containing NaCl as sole salt. Curr Microbiol 22:185–190.

Roden EE, Ferris FG. 2000. Immobilization of aqueous strontium during carbonate formation coupled to microbial Fe(III) oxide reduction. Preprints Ext Abstr ACS Natl Meet, Am Chem Soc, Div Environ Chem 40(2):400–403.
Roemer R, Schwartz W. 1965. Geomikrobiologische Untersuchungen. V. Verwertung von Sulfatmineralien and Schwermetallen. Tolleranz bei Desulfizierern. Z Allg Mikrobiol 5:122–135.
Röttger R. 1974. Large foraminifera: Reproduction and early stages of development in *Heterostigina depressa*. Marine Biol (Berlin) 26:5–12.
Sarbu SM, Kane TC, Kinkle BK. 1996. A chemoautotrophically based cave ecosystem. Science 272:1953–1955.
Sarbu SM, Kinkle, BK, Vlasceanu L, Kane TC, Popa R. 1994. Microbiological characterization of a sulfide-rich groundwater ecosystem. Geomicrobiol J 12:175–182.
Schmalz RF. 1972. Calcium carbonate: Geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, p 110.
Sellwood RW. 1971. The genesis of some sideritic beds in the Yorkshire Lias (England). J Sediment Petrol 41:854–858.
Shachak M, Jones CG, Granot Y. 1987. Herbivory in rocks in the weathering of a desert. Science 236:1098–1099.
Shinano H. 1972a. Studies on marine microorganisms taking part in the precipitation of calcium carbonate. II. Detection and grouping of the microorganisms taking part in the precipitation of calcium carbonate. Bull Jpn Soc Fisheries 38:717–725.
Shinano H. 1972b. Microorganisms taking part in the precipitation of calcium carbonate. III. A taxonomic study of marine bacteria taking part in the precipitation of calcium carbonate. Bull Jpn Soc Sci Fisheries 38:727–732.
Shinano H, Sakai M. 1969. Studies on marine bacteria taking part in the precipitation of calcium carbonate. I. Calcium carbonate deposited in peptone medium prepared with natural seawater and artificial seawater. Bull Jpn Soc Sci Fisheries 35:1001–1005.
Skirrow G. 1975. The dissolved gases—carbon dioxide. In: Riley JR, Skirrow G. Chemical Oceanography, Vol 2. 2nd ed. London: Academic Press, pp 144–152.
Smith SV. 1981. Marine macrophytes as a global carbon sink. Science 211:838–840.
Sokolova-Dubinina GA, Deryugina ZP. 1967. On the role of microorganisms in the formation of rhodochrosite in Punnus-Yarvi Lake. Mikrobiologiya 36:535–542.
Spiro B, Pentecost A. 1991. One day in the life of a stream—A diurnal inorganic carbon mass balance for the travertine depositing stream (Waterfall Beck, Yorkshire). Geomicrobiol J 9:1–11.
Steinmann G. 1899/1901. über die Bildungsweise des dunklen Pigments bei den Mollusken nebst Bemerkungen über die Entstehung von Kalkcarbonat. Ber Naturf Ges Freiburg i B 11:40–45.
Stumm W, Morgan JJ. 1981. Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters. New York: Wiley.
Stutzer A, Hartleb R. 1899. Die Zersetzung von Cement unter dem Einfluss von Bakterien. Z Angew Chem 12:402 (cited by Paine et al., 1933).
Suess E. 1979. Mineral phases formed in anoxic sediments by microbial decomposition of organic matter. Geochim Cosmochim Acta 43:339–352.

Thompson, JB. 2000. Microbial whitings. In: Riding RE, Awramik SM, eds. Microbial Sediments. Berlin: Springer-Verlag, pp 250–260.

Thompson JB, Ferris FG. 1990. Cyanobacterial precipitation of gypsum, calcite and magnesite from natural alkaline lake water. Geology 18:995–998.

Thompson JB, Ferris FG, Smith DA. 1990. Geomicrobiology and sedimentology of the mixolimnion and chemocline in Fayetteville Green Lake, New York. Palaios 5:52–75.

Urzì C, Lisi S, Criseo G, Penrice A. 1991. Adhesion to and decomposition of marble by a *Micrococcus* strain isolated from it. Geomicrobiol J 9:81–90.

Urzì C, Garcia-Valles M, Vendrell M, Pernice A. 1999. Biomineralization processes on rock and monument surfaces observed in field and laboratory conditions. Geomicrobiol J 16:39–54.

van der Wal P, de Jong EW, Westbroek P, de Bruijn WC, Mulder-Stapel AA. 1983. Polysaccharide localization, coccolith formation, and Golgi dynamics in the coccolithophorid *Hymenomonas carterae*. J Ultrastruct Res 85:139–158.

Verrecchia EP, Dumant J-L, Collins KE. 1990. Do fungi building limestone exist in semiarid regions? Naturwissenschaften 77:584–586.

Wagner E, Schwartz W. 1965. Geomikrobiologische Untersuchungen. VIII. über das Verhalten von Bakterien auf der Oberfläche von Gesteinen und Mineralien und ihre Rolle by der Verwitterung. Z Allg Mikrobiol 7:33–52.

Walter MR, ed. 1976. Stromatolites. Developments in Sedimentology 20. Amsterdam: Elsevier.

Warren LA, Maurice PA, Parmar N, Ferris FG. 2001. Microbially mediated calcium carbonate precipitation: Implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants. Geomicrobiol J 18:93–115.

Weast RC, Astle MJ, eds. 1982. CRC Handbook of Chemistry and Physics. 63rd ed. Boca Raton, FL: CRC Press.

Wefer G. 1980. Carbonate production by algae *Halimeda*, *Penicillus*, and *Padina*. Nature (Lond) 285:323–324.

Wharton RA Jr., Parker BC, Simmons GM Jr, Seaburg KS, Love FG. 1982. Biogenic calcite structures in Lake Fryxell, Antarctica. Nature (Lond) 295:403–405.

9

Geomicrobial Interactions with Silicon

9.1 DISTRIBUTION AND SOME CHEMICAL PROPERTIES

The element silicon is one of the most abundant in the Earth's crust, ranking second only to oxygen. Its estimated crustal abundance is 27.7% (wt/wt) whereas that of oxygen is 46.6% (Mitchell, 1955). The concentration of silicon in various components of the Earth's surface is listed in Table 9.1.

In nature, silicon occurs generally in the form of silicates, including aluminosilicates, and silicon dioxide (silica). It is found in primary and secondary minerals. It can be viewed as an important part of the backbone of silicate rock structure. In silicates and aluminosilicates, silicon is usually surrounded by four oxygen atoms in tetrahedral fashion (see Kretz, 1972). In aluminosilicates the aluminum is coordinated with oxygens in tetrahedral or octahedral fashion, depending on the mineral (see Tan, 1986). In clays, which result from weathering of primary aluminosilicates, silica tetrahedral sheets and aluminum hydroxide octahedral sheets are layered in different ways depending on the clay type. In **montmorillonite**-type clays, structural units consisting of single aluminum hydroxide octahedral sheets are sandwiched between two silica tetrahedral sheets. The units are interspaced with layers of water molecules of variable thickness into which other polar molecules, including some organic ones, can enter. This variable water layer allows montmorillonite-type clays to swell. The

TABLE 9.1 Abundances of Silicon on the Earth's Surface

Phases	Concentration	Reference
Granite	336,000 ppm	Bowen, 1979
Basalt	240,000 ppm	Bowen, 1979
Shale	275,000 ppm	Bowen, 1979
Limestone	32,000 ppm	Bowen, 1979
Sandstone	327,000 ppm	Bowen, 1979
Soils	330,000 ppm	Bowen, 1979
Seawater	$3 \times 10^3\ \mu g\ L^{-1}$	Marine Chemistry, 1971
Freshwater	7 ppm	Bowen, 1979

structural units of **illite**-type clays resemble those of montmorillonite-type clays but differ from them in that Al replaces some of the Si in the silica tetrahedral sheets. These substituting Al atoms impart extra charges, which are neutralized by potassium ions between the silica sheets between two successive units. The potassium ions act as bridges that prevent the swelling exhibited by montmorillonite in water. In **kaolinite** clays, structural units consist of silica tetrahedral sheets alternating with aluminum hydroxide octahedral sheets joined to one another by oxygen bridges (see Toth, 1955).

Silicon–oxygen bonds of *siloxane linkages* (Si−O−Si) in silicates and aluminosilicates are very strong [their energy of formation ranges from 3110 to 3142 kcal mol^{-1} or 13,031 to 13,165 kJ mol^{-1}] whereas Al−O bonds are weaker [their energy of formation ranges from 1793 to 1878 kcal mol^{-1} or 7513 to 7869 kJ mol^{-1}]. Bonds between nonframework cations and oxygen are the weakest [energy of formation ranges from 299 to 919 kcal mol^{-1} or 1252 to 3851 kJ mol^{-1}] (values cited by Tan, 1986). The strength of these bonds determines their susceptibility to weathering. Thus Si−O bonds are relatively resistant to acid hydrolysis (e.g., Karavaiko et al., 1984), unlike Al−O bonds. Bonds between cations and oxygen are readily broken by protonation or cation exchange.

Silicate in solution at pH 2–9 exists in the form of undissociated monosilicic acid (H_4SiO_4), whereas at pH 9 and above it transforms into silicate ions (see Hall, 1972). Monosilicic acid polymerizes at a concentration of 2×10^{-3} M and above, forming oligomers of polysilicic acids (Iler, 1979). This polymerization reaction appears to be favored around neutral pH (Avakyan et al., 1985). Polymerization of monosilicate can be viewed as a removal of water from between adjacent silicates to form a siloxane linkage. **Silica** can be viewed as an anhydride of *silicic acid*:

$$H_4SiO_4 \rightarrow SiO_2 + 2H_2O \tag{9.1}$$

Dissociation constants for silicic acid are as follows (see Anderson, 1972):

$$H_4SiO_4 \rightarrow H^+ + H_3SiO_4^- \qquad (K_1 = 10^{-9.5}) \tag{9.2}$$

$$H_3SiO_4^- \rightarrow H^+ + H_2SiO_4^{2-} \qquad (K_2 = 10^{-12.7}) \tag{9.3}$$

Silica can exist in partially hydrated form called *metasilicic acid* (H_3SiO_3) or in a fully hydrated form called orthosilicic acid (H_4SiO_4). Each of these forms can be polymerized, the ortho acid forming, for instance, $H_3SiO_4 \cdot H_2SiO_3 \cdot H_3SiO_3$ (Latimer and Hildebrand, 1940; Liebau, 1985). The polymers may exhibit colloidal properties, depending on size and other factors. Colloidal forms of silica tend to exist locally at high concentrations or at saturation levels and are favored by acid conditions (Hall, 1972).

Common silicon-containing minerals include quartz (SiO_2), olivine [$(Mg,Fe)_2SiO_4$], orthopyroxene ($Mg,FeSiO_3$), biotite [$K(Mg,Fe)_3AlSi_3O_{10}(OH)_2$], orthoclase ($KAlSi_3O_8$), plagioclase ($Ca,Na)(Al,Si)AlSi_2O_8$], kaolinite [$Al_4Si_4O_{10}(OH)_8$], and others.

Silica and silicates form an important buffer system in the oceans (Garrels, 1965), together with the $CO_2/HCO_3^-/CO_3^{2-}$ system. The latter is a rapidly reacting system, whereas the system based on reaction with silica and silicates is slow (Garrels, 1965; Sillén, 1967).

Aluminosilicates in the form of clay perform a buffering function in mineral soils. This is because of their ion-exchange capacity, their net electronegative charge, and their adsorption powers. Their ion-exchange capacity and adsorption powers, moreover, make them important reservoirs of cations and organic molecules. Montmorillonite exhibits the greatest ion-exchange capacity, illites exhibit less, and kaolinites the least (Dommergues and Mangenot, 1970, p. 469).

9.2 BIOLOGICALLY IMPORTANT PROPERTIES OF SILICON AND ITS COMPOUNDS

Silicon is taken up and concentrated in significant quantities by certain forms of life. These include microbial forms, such as diatoms and other chrysophytes; silicoflagellates and some xanthophytes; radiolarians and actinopods; some plants such as horsetails, ferns, grasses, and some flowers and trees; and also some animals such as sponges, insects, and even vertebrates. Some bacteria (Heinen, 1960) and fungi (Heinen, 1960; Holzapfel and Engel, 1954a, 1954b) have also been reported to take up silicon to a limited extent. According to Bowen (1966), diatoms may contain from 1500 to 20,000 ppm of silicon, land plants from 200 to 5000 ppm, and marine animals from 120 to 6000 ppm.

Although the function of silicon in most higher forms of life, animals, and plants is not presently apparent, it is clearly structural in some microorganisms,

such as diatoms, actinopods, and radiolarians. In diatoms, silicon also seems to play a metabolic role in the synthesis of chlorophyll (Werner, 1966, 1967), the synthesis of DNA (Darley and Volcani, 1969; Reeves and Volcani, 1984), and the synthesis of DNA polymerase and thymidylate kinase (Sullivan, 1971; Sullivan and Volcani, 1973).

Silicon compounds in the form of clays (aluminosilicates) may exert an effect on microbes in soil. They may stimulate or inhibit microbial metabolism, depending on conditions (e.g., Marshman and Marshall, 1981a, 1981b; Weaver and Dugan, 1972; see also the discussion by Marshall, 1971). These effects of clays are mostly indirect, i.e., clays tend to modify the microbial habitat physicochemically, thereby eliciting a physiological response by the microbes (Stotzky, 1986). For beneficial effect, clays may buffer the soil environment and help to maintain a favorable pH, thereby promoting growth and metabolism of some microorganisms that might otherwise be slowed or stopped if the pH became unfavorable (Stotzky, 1986). Certain clays have been found to enable some bacteria that were isolated from marine ferromanganese nodules or associated sediments to oxidize Mn^{2+}. These microorganisms can oxidize Mn^{2+} if it is bound to bentonite (a montmorillonite-type clay) or kaolinite but not illite if each has been pretreated with ferric iron. They cannot oxidize Mn^{2+} that is free in solution (Ehrlich, 1982). Cell-free preparations of these bacteria oxidize Mn^{2+} bound to bentonite and kaolinite even without ferric iron pretreatment, although the manganese-oxidizing activity of the cell extracts is greater when the clays are pretreated with ferric iron (Ehrlich, 1982). Like intact cells, the cell-free extract cannot oxidize Mn^{2+} free in solution (Ehrlich, 1982). Clays may also enhance activity of some enzymes such as catalase when the enzymes are bound to their surface (see Stotzky, 1986, p. 404).

By contrast, clays may suppress microbial growth and metabolism by adsorbing microbial nutrients, thereby making them less available to microbes. Clays may also adsorb microbial antibiotics and thereby lower the inhibitory activity of these agents (see Stotzky, 1986). In soils the results may be that an antibiotic producer is outgrown by organisms it normally keeps in check with the help of the antibiotic. These effects of clay can be explained, at least in part, by the strength of binding to a negatively charged clay surface and the inability of many microbes to attack adsorbed nutrients or by the inability of adsorbed antibiotics to inhibit susceptible microbes (see, e.g., Dashman and Stotzky, 1986). High concentrations of clay may interfere with diffusion of oxygen by increasing the viscosity of a solution, which can have a negative effect on aerobic microbial respiration (see Stotzky, 1986). Clays may also modulate other interactions between different microbes and between microbes and viruses in soil, and they may affect the pathogenicity of disease-causing soil microbes (see Stotzky, 1986).

Although clay-bound organic molecules may be less available or unavailable to organisms in the bulk phase or even attached to the mineral surface, this

cannot be a universal phenomenon. Portions of attached large organic polymers may be attacked by appropriate extracellular enzymes, producing smaller units that can be taken up by microbes. Electrostatically bound organic molecules that are potential nutrients may be dislodged by exchange with protons excreted as acids in the catabolism of some microbes. These processes of remobilization must also apply to mineral sorbents other than clays.

9.3 BIOCONCENTRATION OF SILICON

Bacteria

Some bacteria have been shown to accumulate silicon. A soil bacterium, strain B_2 (Heinen, 1960), and a strain of *Proteus mirabilis* (Heinen, 1968) have been found to take up limited amounts of silicon when it is furnished in the form of silica gel, quartz, or sodium silicate. Sodium silicate was taken up most easily, quartz least easily. The silicon seemed to substitute partially for phosphorus in phosphorus-deficient media (Fig. 9.1). This substitution reaction was reversible (Heinen, 1962). The silicon taken up by the bacteria appeared to become organically bound in a metabolizable form (Heinen, 1962). Sulfide, sulfite, and sulfate were found to affect phosphate–silicate exchange in different ways, depending on concentration, whereas KCl and NaCl were without effect. NH_4Cl, NH_4NO_3, and $NaNO_3$ stimulated the formation of adaptive (inducible) enzymes involved in the phosphate–silicate exchange (Heinen, 1963a). The presence of sugars such as glucose, fructose, or sucrose and of amino acids such as alanine, cysteine, glutamine, methionine, asparagine, and citrulline as well as of metabolic intermediates pyruvate, succinate, and citrate stimulated silicon uptake. On the other hand, acetate, lactate, phenylalanine, peptone, and wheat germ oil inhibited silicon uptake. Glucose at an initial concentration of $1.2\,mg\,mL^{-1}$ of medium stimulated silicon uptake maximally (Fig. 9.2). Higher concentrations of glucose caused the formation of particles of protein, carbohydrates, and silicon outside the cell. $CdCl_2$ inhibited the stimulatory effect of glucose on silicon uptake, but 2,4-dinitrophenol was without effect. The simultaneous presence of $NaNO_3$ and KH_2PO_4 lowered the stimulatory effect of glucose but did not eliminate it (Heinen, 1963b). The silicon that was fixed in the bacteria was readily displaced by phosphate in the absence of external glucose. In cells that were preincubated in a glucose–silicate solution, only a small portion of the silicon was released by glucose–phosphate, but all of the silicon was releasable on incubation in glucose–carbonate solution.

Some of the silicon taken up by the bacterial cells appeared to be tied up in labile ester bonds (C−O−Si), whereas other silicon appeared to be tied up in more stable bonds (C−Si) (Heinen, 1963c, 1965). Studies of intact cells and cell extracts of *Proteus mirabilis* after the cells were incubated in the presence of

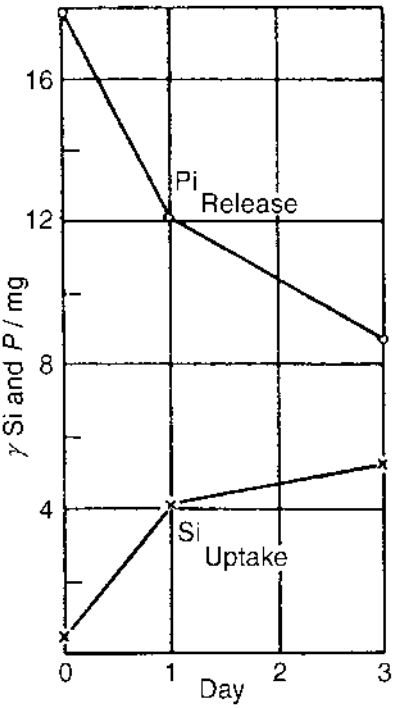


FIG. 9.1 Relationship between Si uptake and P_i release during incubation of resting cells of strain B_2 in silicate solution (80 μg Si mL^{-1}). (From Heinen W., Silicium-Stoffwechsel bei Mikroorganismen. II. Mitteilung. Beziehung zwischen dem Silica und Phosphat-Stoffwechsel bei Bakterien. Arch Mikrobiol 41:229–246, Copyright 1962, Springer-Verlag, with permission.)

silicate suggested that the silicate taken up was first accumulated in the cell walls and then slowly transferred to the interior of the cell (Heinen, 1965). The silicon was organically bound in the wall and within the cell. A particulate fraction from *P. mirabilis* bound silicate organically in an oxygen-dependent process (Heinen, 1967).

Fungi

Some fungi have also been reported to accumulate silicon (Holzapfel and Engel, 1954a, 1954b). When growing on a silicate-containing agar medium, the vegetative mycelium of such fungi exhibits an induction period of 5–7 days before taking up silicon. When the silicon in the medium is in the form of galactose– or glucose–quartz complexes, silicon uptake by vegetative mycelium can occur within 12–18 hr (Holzapfel, 1951). Evidently, inorganic silicates have

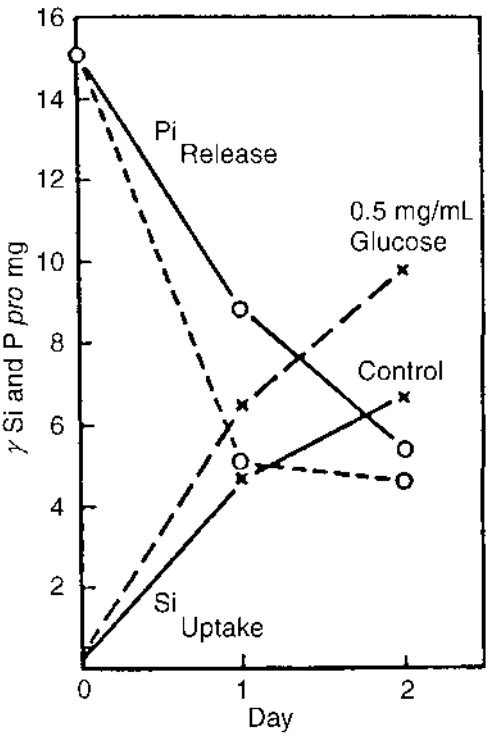


FIG. 9.2 Influence of glucose and silicate uptake (x—x, without glucose, control; x---x, with glucose), and phosphate release (o—o, without glucose; o---o, with glucose). (From Heinen W. Silicium-Stoffwechsel bei Mikroorganismen. IV. Mitteilung. Die Wirkung zwischen organischer Verbindungen, insbesondere Glucose, auf den Stoffwechsel bei Bakterien. Arch Mikrobiol 45:162–171, Copyright 1963, Springer-Verlag, with permission.)

to be transformed into organic complexes during the prolonged induction period before the silicon is taken into the fungal cells.

Diatoms

Among eukaryotic microorganisms that take up silicon, the diatoms have been most extensively studied with respect to this property (Fig. 9.3) (Lewin, 1965; de Vrind-de Jong and de Vrind, 1997). Their silicon uptake ability affects redistribution of silica between fresh and marine waters. In the Amazon River estuary, for instance, diatoms remove 25% of the dissolved silica from the river water. Their frustules are not swept oceanward upon their death but are transported coastward and incorporated into dunes, mud, and sandbars (Millman and Boyle, 1975).

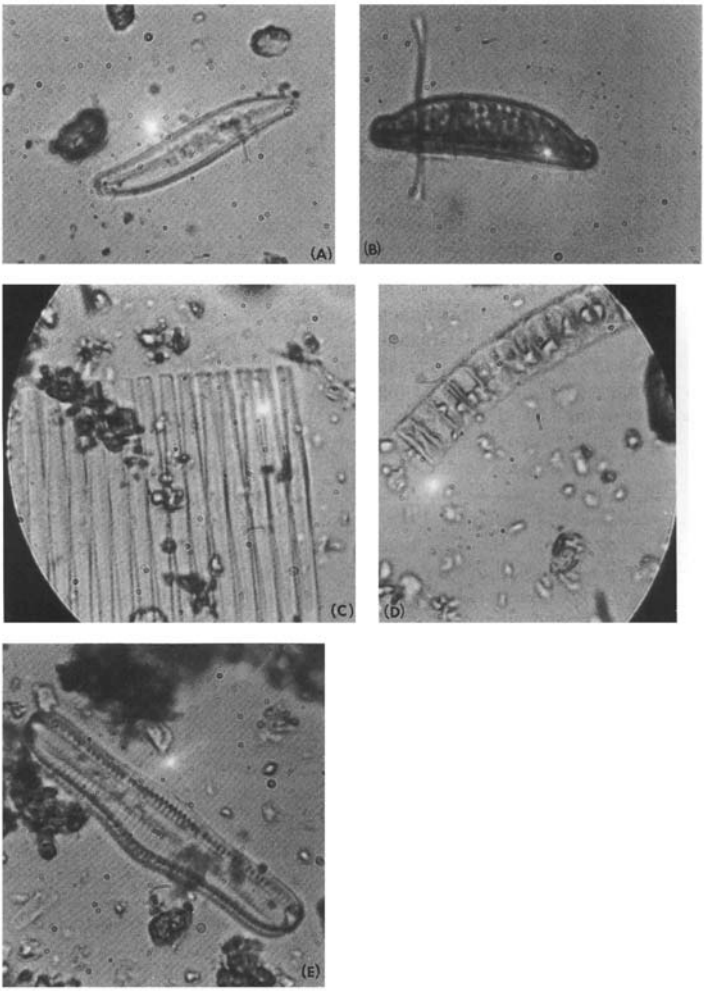


FIG. 9.3 Diatoms. (A) *Gyrosigma*, from freshwater (×1944); (B) *Cymbella* from freshwater (×1944); (C) *Fragellaria*, from freshwater (×1864); (D) ribbon of diatoms, from freshwater (×1990); (E) marine diatom frustule, from Pacific Ocean sediment (×1944).

Diatoms are unicellular algae enclosed in a wall of silica consisting of two valves, an epivalve and a hypovalve, in pillbox arrangement. One or more girdle bands are loosely connected to the epivalve. The valves are usually perforated plates, which may have thickened ribs. Their shape may be pennate or centric. The pores serve as sites of gas and nutrient exchange (see de Vrind-de Jong and

de Vrind, 1997). In cell division, each daughter cell receives either the epivalve or the hypovalve of the mother cell and synthesizes the other valve de novo to fit into the one already present. To prevent excessive reduction in size of daughter diatoms that receive the hypovalve after each cell division, a special reproductive step called *auxospore formation* returns these daughter cells to maximum size. It occurs when a progeny cell that has received a hypovalve has reached minimum size after repeated divisions. Auxospore formation is a sexual reproductive process in which the cells escape from their frustules and increase in size in their zygote membrane, which may become weakly silicified. After a time, the protoplast in the zygote membrane contracts and forms the typical frustules of the parent cell (Lewin, 1965).

The silica walls of diatoms consist of hydrated amorphous silica, a polymerized silicic acid (Lewin, 1965). The walls of marine diatoms may contain as much as 96.5% SiO_2 but only 1.5% Al_2O_3 or Fe_2O_3 and 1.9% water (Rogall, 1939). In clean, dried frustules of freshwater *Navicula pelliculosa*, 9.6% water has been found (Lewin, 1957). Thin parts of diatom frustules reveal a foamlike substructure under the electron microscope, suggesting silica gel (Helmcke, 1954), which may account for the adsorptive power of such frustules. The silica gel may be viewed as arranged in small spherical particles about 22 μm in diameter (Lewin, 1965). Because of the low degree of solubility of amorphous silica at the pH of most natural waters, frustules of living diatoms do not dissolve readily (Lewin, 1965). At pH 8, however, it has been found that 5% of the silica in the cell walls of *Thalassiosira nana* and *Nitschia linearis* dissolves. Moreover, at pH 10, 20% of the silica in the frustules of *N. linearis* and all of the silica in the frustules of *T. nana* dissolves (Jorgensen, 1955). This silica dissolution may reflect the state of integration of newly assimilated silica in the diatom frustule. Some bacteria naturally associated with diatoms have been shown to accelerate dissolution of silica in frustules by an unknown mechanism (Partick and Holding, 1985). Diatom frustules of living organisms are to some extent protected against dissolution by an organic film, when present, and their rate of dissolution has been shown to exhibit temperature dependence (Katami, 1982). After the death of diatoms, their frustules may dehydrate to form more crystalline SiO_2, which is much less soluble in alkali than that in living diatoms. This may account for the accumulation of diatomaceous ooze.

Rates of silicic acid uptake and incorporation by diatoms can be easily measured with radioactive [^{68}Ge]germanic acid as tracer (Azam et al., 1973; Azam, 1974; Chisholm et al., 1978). At low concentration (Ge/Si molar ratio of 0.01), germanium, which is chemically similar to silicon, is apparently incorporated together with silicon into the silicic acid polymer of the frustule. At higher concentrations (Ge/Si molar ratio of 0.1), germanium is toxic to diatoms (Azam et al., 1973).

Diatoms are able to discriminate between ^{28}Si and ^{30}Si by assimilating the lighter isotope preferentially. The fractionation factor (α) for each of the three

diatom species *Skeletonema costatum*, *Thalassiosira weissflogii*, and *Thalassiosira* sp. was 0.9989±0.004. It was independent of growth temperature between 12 and 22°C and thus independent of growth rate (De La Rocha et al., 1997). This fractionation ability appears to be usable as a signature in identifying biogenic silica (De La Rocha et al., 2000).

Diatoms take up silica in the form of orthosilicate. More highly polymerized forms of silicate are not taken up unless first depolymerized, as by some bacteria (Lauwers and Heinen, 1974). Organic silicates are also not available to them. Ge, C, Sn, Pb, P, As, B, Al, Mg, and Fe do not replace silicon extensively if at all (Lewin, 1965). The concentration of silicon accumulated by a diatom depends to some extent on its concentration in the growth medium and on the rate of cell division (the faster the cells divide, the thinner their frustules). Silicon is essential for cell division, but resting cells in a medium in which silica is not at a limiting concentration continue to take up silica (Lewin, 1965). Synchronously growing cells of *Navicula pelliculosa* take up silica at a constant rate during the cell division cycle (Lewin, 1965). Silica uptake appears dependent on energy-yielding processes (Lewin, 1965; Azam et al., 1974; Azam and Volcani, 1974) and seems to involve intracellular receptor sites (Blank and Sullivan, 1979). Uncoupling of oxidative phosphorylation stops silica uptake by *Navicula pelliculosa* and *Nitschia angularis*. Starved cells of *Navicula pelliculosa* show an enhanced silica uptake rate when fed glucose or lactate in the dark or when returned to the light, where they can photosynthesize (Healy et al., 1967). Respiratory inhibitors prevent Si and Ge uptake by *Nitschia alba* (Azam et al., 1974; Azam and Volcani, 1974). Total uptake of phosphorus and carbon is decreased during silica starvation of *Navicula pelliculosa*. Upon restoration of silica to the medium, the total uptake of phosphorus and carbon is again increased (Coombs and Volcani, 1968). Sulfhydryl groups appear to be involved in silica uptake (Lewin, 1965).

Some progress has been made in understanding how diatoms form their siliceous cell walls (see de Vrind-de Jong and de Vrind, 1997). Valve and girdle-band assembly takes place inside the cell and happens late in the cell cycle during the last part of mitosis. For this assembly, silicate is taken into the cell and polymerized in special membrane-bound silica deposition vesicles (SDVs), leading to the formation of the girdle bands and valves. The SDV seems to arise from the Golgi apparatus, a special membrane system within the cell. The endoplasmic reticulum, a membrane network inside the cell that is connected to the plasma membrane and the nuclear membrane, may participate in SDV development. The active SDVs are located adjacent to the plasma membrane. The shape of an SDV may be determined by interaction of various cell components, such as the plasma membrane, actin filaments, microtubules, and cell organelles. The SDV is believed to help determine the morphology of the valves. Frustule buildup in the SDV appears to start along the future raphe, which

appears as a longitudinal slot in each mature frustule. The raphe has a central thickening called a nodule. Completed valves are exocytosed by the cell, that is, they are exported to the cell surface. When the valves are in place at the cell surface of the diatom, the raphe plays a role in its motility. Mature frustules have glycoprotein associated with them, which may have played a role in silica assembly during valve formation. It may help in determining valve morphology and in the export of assembled valves to the cell surface. For additional information, the reader is referred to de Vrind-de Jong and de Vrind (1997) and references cited therein.

9.4 BIOMOBILIZATION OF SILICON AND OTHER CONSTITUENTS OF SILICATES (BIOWEATHERING)

Some bacteria and fungi play an important role in mobilization of silica and silicates in nature. Part of this microbial involvement is manifested in the weathering of rock silicates and aluminosilicates. The solubilizing action may involve the cleavage of Si—O—Si (siloxane) or Al—O framework bonds or the removal of cations from the crystal lattice of silicate, causing a subsequent collapse of the silicate lattice structure. The mode of attack may be by (1) microbially produced ligands of cations, (2) microbially produced organic or inorganic acids, which are a source of protons, (3) microbially produced alkali (ammonia or amines), or (4) microbially produced extracellular polysaccharide acting at acid pH. The source of the polysaccharides may be the glycocalyx of some bacteria.

Bioweathering action of silica and silicates seems not to be restricted to corrosive agents that have been excreted by appropriate microorganisms into the bulk phase but can also involve microbes attached to the surface of silica or silicates (e.g., Bennett et al., 1996, 2001). Because they are attached, their excreted metabolic products can attack the mineral surface in more concentrated form. Such attack may be manifested in etch marks. Some of the polysaccharides by which the microbes attach to the mineral surface may themselves be corrosive.

Bioweathering, like abiotic weathering, can lead to the formation of new minerals. This is the result of reprecipitation and crystallization of some of the mobilized constituents from the mineral being weathered (e.g., Barker and Banfield, 1996; Adamo and Violante, 2000). The new, secondary minerals may form on the surface of the weathered mineral. Microbes attached to the surface of minerals being weathered may serve as nucleating agents in mineral neoformation (e.g., Schultze-Lam et al., 1996; Macaskie et al., 1992).

Solubilization by Ligands

Microbially produced ligands of divalent cations have been shown to cause dissolution of calcium-containing silicates. For instance, a soil strain of *Pseudomonas* that produced 2-ketogluconic acid from glucose dissolved synthetic silicates of calcium, zinc, and magnesium and the minerals wollastonite ($CaSiO_3$), apophyllite [$KCa_4Si_8O_{20}(F,OH) \cdot 8H_2O$], and olivine [$(Mg,Fe)_2SiO_4$] (Webley et al., 1960). The demonstration consisted of culturing the organism for 4 days at 25 °C on separate agar media, each containing 0.25% (wt/vol) of one of the synthetic or natural silicates, which rendered the medium turbid. A clear zone was observed around the bacterial colonies when silicate was dissolved (Fig. 9.4). A similar silicate-dissolving action with a gram-negative bacterium, strain D_{11}, which resembled *Erwinia* spp., and with *Bacterium* (now *Erwinia*) *herbicola* or with some *Pseudomonas* strains, all of which were able to produce 2-keto-

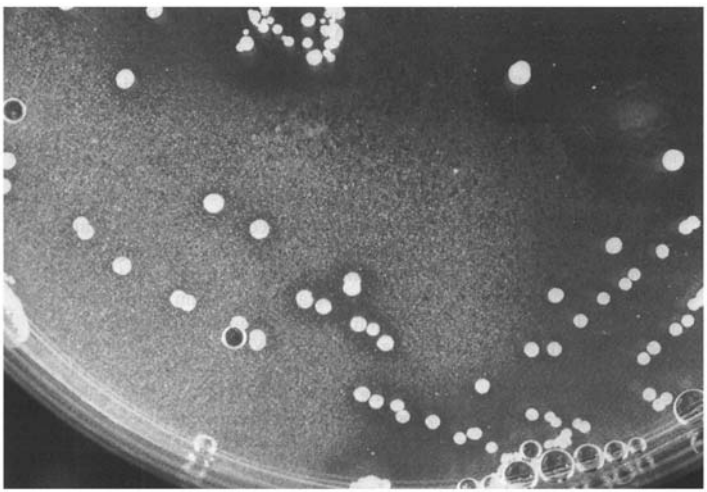


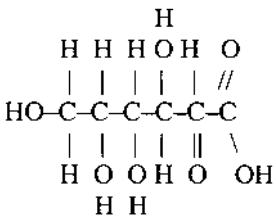
FIG. 9.4 Colonies of bacterial isolate C-2 from a sample of weathered rock on synthetic calcium silicate selection medium showing evidence of calcium silicate dissolution around colonies. Basal medium was prepared by aseptically mixing 10 mL of sterile solution A (3 g dextrose or 3 g levulose in 100 mL distilled water), 10 mL of sterile solution B [0.5 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g Na_2HPO_4, 0.5 g KH_2PO_4, 2 g yeast extract, 0.05 g $MnSO_4 \cdot H_2O$ in 500 mL distilled water], and 20 mL of sterile 3% agar and distributing the mixture in Petri plates. Capping agar was prepared by mixing 10 mL of a sterile synthetic $CaSiO_3$ suspension with 7.5 mL of sterile solution A, 7.5 mL of sterile solution B, and 15 mL of sterile 3% agar and distributing 3 mL of this mixture aseptically over the surface of the solidified basal agar in the plates.

gluconate from glucose, was also shown (Duff et al., 1963). The action of these bacteria was tested in glucose-containing basal medium: KH_2PO_4, 0.54 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $(NH_4)_2SO_4$, 0.75 g; $FeCl_3$, trace; Difco yeast extract, 2 g; glucose, 40 g; distilled water, 1 L; and 5–500 mg pulverized mineral per 5–10 mL of medium. It was found that dissolution of silicates in these cases resulted from the complexation of the cationic components of the silicates by 2-ketogluconate. The complexes were apparently more stable than the silicates. For example,

$$CaSiO_3 \Leftrightarrow Ca^{2+} + SiO_3^{2-} \tag{9.4}$$

$$Ca^{2+} + \text{2-ketogluconate} \rightarrow \text{Ca(2-ketogluconate)} \tag{9.5}$$

The structure of 2-ketogluconic acid is



The silicon that was liberated or released in these experiments and subsequently transformed took three forms. (1) low molecular weight or ammonium molybdate reactive silicate (monomeric?); (2) a colloidal polymeric silicate of higher molecular weight, which did not react with dilute hydrofluoric acid; and (3) an amorphous form that could be removed from solution by centrifugation and dissolved in cold 5% aqueous sodium carbonate (Duff et al., 1963). Polymerized silicate can be transformed by bacteria into monomeric silicate, as has been shown in studies with *Proteus mirabilis* and *B. caldolyticus* (Lauwers and Heinen, 1974). The *Proteus* culture was able to assimilate some of the monomeric silicate. The mechanism of depolymerization has not been elucidated. It may involve an extracellular enzyme.

Gluconic acid produced from glucose by several different types of bacteria has been shown to solubilize bytownite, albite, kaolinite, and quartz at near-neutral pH (Vandevivere et al., 1994). The activity around neutral pH suggests that the mechanism of action of gluconate involves chelation.

Quartz (SiO_2) has been shown to be subject to slow dissolution by organic acids such as citric, oxalic, pyruvic, and humic acids (Bennett et al., 1988), all of which can be formed by fungi and bacteria. In a pH range of 3–7, the effect was

greatest at pH 7, indicating that the mechanism of action was not acidulation but chelation. Bennett et al. (1988) suggest that the chelation involves an electron donor–acceptor system. Acetate, fumarate, and tartrate were ineffective in dissolving silica as a complex.

Solubilization by Acids

The effect of acids in solubilizing silicates has been noted in various studies. Waksman and Starkey (1931) cited the action of CO_2 on orthoclase:

$$2KAlSi_3O_8 + 2H_2O + CO_2 \rightarrow H_4Al_2Si_2O_9 + K_2CO_3 + 4SiO_2 \qquad (9.6)$$

The CO_2 is, of course, very likely to be a product of respiration or fermentation. Its weathering action is best viewed as based on the formation of the weak acid H_2CO_3 through hydration of the CO_2. Another example of a silicate attack by acid is that involving spodumene ($LiAlSi_2O_6$) (Karavaiko et al., 1979). In this instance, an in situ correlation was observed between the degree of alteration of a spodumene sample and the acidity it produced when in aqueous suspension. Unweathered spodumene generated a pH in the range of 5.1–7.5, whereas altered spodumene generated a pH in the range of 4.2–6.4. Non-spore-forming heterotrophs were found to predominate in weathered spodumene. They included bacteria such as *Arthrobacter pascens*, *A. globiformis*, and *A. simplex* as well as *Nocardia globerula*, *Pseudomonas fluorescens*, *P. putida*, and *P. testeronii* and fungi such as *Trichoderma lignorum*, *Cephalosporum atrum*, and *Penicillium decumbens*. Acid decomposition of spodumene may be formulated as follows (Karavaiko et al., 1979):

$$4LiAlSi_2O_6 + 6H_2O + 2H_2SO_4 \rightarrow 2Li_2SO_4 + Al_4(Si_4O_{10})(OH)_8 + 4H_2SiO_3 \qquad (9.7)$$

The aluminosilicate product in this reaction is kaolinite.

Further investigation into microbial spodumene degradation revealed that among the most active microorganisms are the fungi *Penicillium notatum* and *Aspergillus niger*, thionic bacteria like *Thiobacillus thiooxidans*, and the slime-producing bacterium *Bacillus mucilaginosus* var. *siliceus* (Karavaiko et al., 1980; Avakyan et al., 1986). The fungi and *T. thiooxidans*, which produce acid, were most effective in solubilizing Li and Al. *B. mucilagenosus* was effective in solubilizing Si in addition to Li and Al by reaction of its extracellular polysaccharide with the silicate in spodumene.

Solubilization of silicon along with other constituents in the primary minerals amphibolite, biotite, and orthoclase by acids (presumably citric and oxalic acids) formed by several fungi and yeasts at the expense of glucose has also been demonstrated (Eckhardt, 1980; see also Barker et al., 1997). These

findings of silicon mobilization are similar to those in earlier studies on the action of the fungi *Botritis*, *Mucor*, *Penicillium*, and *Trichoderma* isolated from rock surfaces and weathered stone. In these experiments, citric and oxalic acids produced by the fungi solubilized Ca, Mg, and Zn silicates (Webley et al., 1963). *Aspergillus niger* has been shown to release Si from apophyllite, olivine, saponite, vermiculite, and wollastonite, but not augite, garnet, heulandite, hornblende, hypersthene, illite, kaolinite, labradorite, orthoclase, or talc in studies by Henderson and Duff (1963). However, Berner et al. (1980) found in laboratory experiments that augite, hypersthene, hornblende, and diopside in soil samples were subject to weathering by soil acids, presumably of biological origin. Organisms different from those used by Henderson and Duff (1963) and, as a result, different metabolic products were probably involved. *Penicillium simplicissimus* released Si from basalt, granite, grandiorite, rhyolite, andesite, peridotite, dunite, and quartzite with metabolically produced citric acid (Silverman and Munoz, 1970). Acid formed by *Penicillium notatum* and *Pseudomonas* sp. released Si from plagioclase and nepheline (Aristovskaya and Kutuzova, 1968; Kutuzova, 1969).

In a study of weathering by organic and inorganic acids of three different plagioclase specimens (Ca-Na feldspars), it was found that steady-state dissolution rates were highest at approximately pH 3 and decreased as the pH was increased toward neutrality (Welch and Ullman, 1993). The organic and inorganic acids whose weathering action was studied are representative of some end products of microbial metabolism. Polyfunctional organic acids, including oxalate, citrate, succinate, pyruvate, and 2-ketoglutarate, were the most effective, whereas acetate and propionate were less effective. However, all organic acids were more effective than the inorganic acids HCl and HNO_3. The polyfunctional acids acted mainly as acidulants at very acid pH and mainly as chelators near neutral pH. Ullman et al. (1996) found that in some instances the combined effect of protonation and chelation enhanced the solubilizing action of some polyfunctional acids on feldspars by a factor of 10 above the expected proton-promoted rate. Ca and Na were rapidly released in these experiments. The chelate attack appeared to be at the Al sites. Those organic acids that preferentially chelated Al were the most effective in enhancing plagioclase dissolution. Although the products of dissolution of feldspars are usually considered to include separate aluminum and silicate species, soluble aluminum–silicate complexes may be intermediates (Browne and Driscoll, 1992).

The practical effect of acid attack of aluminosilicates can be seen in the corrosion of concrete sewer pipes. Concrete is formed from a mixture of cement (heated limestone, clay, and gypsum) and sand. On setting, the cement includes the compounds Ca_2SiO_4, Ca_3SiO_5, and $Ca_3(AlO_3)_2$, which hold the sand in their matrix. H_2S produced by microbial mineralization of organic sulfur compounds and by bacterial reduction of sulfate in sewage can itself corrode concrete. But

corrosion is enhanced if the H_2S is first oxidized to sulfuric acid by thiobacilli (*T. neapolitanus*, *T. intermedius*, *T. novellus*, *T. thiooxidans*) (Parker, 1947; Milde et al., 1983; Sand and Bock, 1984).

Groundwater pollution with biodegradable substances has been found to result in silicate weathering of aquifer rock. Products of microbial degradation of these substances cause the weathering. This was observed in an oil-polluted aquifer near Bemidji, Minnesota (Hiebert and Bennett, 1992). Microcosm experiments of 14 months duration in the aquifer with a mixture of crystals of albite, anorthite, anorthoclase, microcline, each of which is a feldspar mineral, and quartz revealed microbial colonization of the mineral surfaces by individual cells and small clusters. Intense etching of the feldspar minerals and light etching of the quartz occurred at or near where the bacteria were seen. Such aquifer rock weathering can affect water quality.

Solubilization by Alkali

Alkaline conditions are very conducive to mobilizing silicon, whether from silicates, aluminosilicates, or even quartz (Karavaiko et al., 1984). This is because both the Al−O and Si−O bonds are very labile under these conditions, because both are susceptible to nucleophilic attack (see discussion by Karavaiko et al., 1984). *Sarcina ureae* growing in a peptone–urea broth released silicon readily from nepheline, plagioclase, and quartz (Aristovskaya and Kutuzova, 1968; Kutuzova, 1969). In this instance, ammonia resulting from the hydrolysis of urea was the source of alkali. In microbial spodumene degradation, alkaline pH also favors silicon release (Karavaiko et al., 1980).

Pseudomonas mendocina is able to enhance mobilization of Al, Si, and Fe impurities from kaolinite in a succinate–mineral salts medium in which the pH rose from ~7.7 to 9.2 in 4 days of growth under aerobic conditions (Maurice et al., 2001).

Solubilization by Extracellular Polysaccharide

Extracellular polysaccharide has been claimed to play an important role in silicon release, especially in the case of quartz. Such polysaccharide is able to react with siloxanes to form organic siloxanes. It can be of bacterial origin [e.g., from *Bacillus mucilagenosus* var. *siliceus* (Avakyan et al., 1986) or unnamed organisms in a microbial mat on rock around a hot spring (Heinen and Lauwers, 1988)] or of fungal origin (e.g., from *Aspergillus niger*) (Holzapfel and Engel, 1954b). The reaction appears not to be enzyme-catalyzed, because polysaccharide from which the cells have been removed is reactive. Indeed, such organic silicon-containing compounds can be formed with reagent grade organics (Holzapfel, 1951; Weiss et al., 1961) and have been isolated from various biological sources

other than microbes (Schwarz, 1973). With polysaccharide from *Bacillus mucilaginosus*, the reaction appears to be favored by acid metabolites (Malinovskaya et al., 1990). Welch and Vandevivere (1995) found that polysaccharides from different sources either had no effect or interfered with solubilization of plagioclase by gluconate at a pH between 6.5 and 7.0.

Barker and Banfield (1996) described the weathering of amphibole syenite associated with the Stettin complex near Wausau, Wisconsin, by bacteria and lichens. The process involved penetration of grain boundaries, cleavages, and cracks. Mineral surfaces became coated with acid mucopolysaccharides (biofilm formation?). In the weathering, dissolution by metabolically produced corrosive agents and selective transport of mobilized constituents, likely mediated by acid mucopolysaccharides, occurred. Some mobilized constituents reprecipitated, leading to the formation of clay minerals.

A more detailed study revealed that the site of bioweathering by lichen (a symbiotic consortium of a fungus and an alga) could be divided into four zones (Baker and Banfield, 1998). In the uppermost zone (zone 1), represented by the upper lichen thallus, no weathering occurs. This is the photosynthetic zone. In zone 2, involving the lower lichen thallus, active weathering due to interaction with lichen products occurs. Mineral fragments coated with organic polymers of incipient secondary minerals that resulted from the weathering may appear in the thallus. In zone 3, reactions occur that are an indirect consequence of lichen action. In zone 4, any weathering, if it occurs, is due to abiotic processes.

Depolymerization of Polysilicates

Because silicate can exist in monomeric form as well as polymeric form (metasilicates, siloxanes), and because silicon uptake by microbes depends on the monomeric form (orthosilicate), depolymerization of siloxanes is important. *Proteus mirabilis* and *Bacillus caldolyticus* have the capacity to promote this process. In the case of *B. caldolyticus*, it appears to be growth-dependent even though the organism does not assimilate Si (Lauwers and Heinen, 1974). In the weathering of quartz, the degradation of the mineral to the monomeric stage appears to proceed through an intermediate oligomeric stage. Organosilicates may also be formed transitionally (Avakyan et al., 1985). The detailed mechanism by which these transformations proceed is not known. It is clear, however, that these biodegradative processes are of fundamental importance to the biological silica cycle.

9.5 ROLE OF MICROBES IN THE SILICON CYCLE

As the foregoing discussion shows, microbes [even some plants and animals (Drever, 1994)] have a significant influence on the distribution and form of silicon

in the biosphere. Those organisms that assimilate silicon clearly act as concentrators of it. Those that degrade silica, silicates, or aluminosilicates act as agents of silicon dispersion. They are an important source of orthosilicate on which the concentrators depend, and they are also important agents of rock weathering. Comparative electron microscopic studies have provided clues to the extent of microbial weathering action (see, e.g., Berner et al., 1980; Callot et al., 1987; Barker and Banfield, 1998).

It has been argued that silicate-liberating reactions by microbially produced acids and complexing agents under laboratory conditions occurred at glucose concentrations that may not be encountered in nature and may therefore be laboratory artifacts. A counterargument can be made, however, that microenvironments exist in soil and sediment that have appropriately high concentrations of utilizable carbohydrates, nitrogenous compounds, and other needed nutrients. They originate from the excretory products and the dead remains of organisms from which appropriate bacteria and/or fungi can form the compounds that can promote the breakdown of quartz, silicates, and aluminosilicates. Indeed, fungal hyphae in the litter zone and A horizon of several different soils have been shown by scanning electron microscopy to carry calcium oxalate crystals attached to them. This is evidence for extensive in situ production of oxalate by the fungus (Graustein et al., 1977). The basidiomycete *Hysterangium crassum* was shown to weather clay in situ with the oxalic acid it produced (Cromack et al., 1979). Lichens show evidence, observable in situ, of extensive rock weathering activity (Jones et al., 1981.) Although Ahmadjian (1967) and Hale (1967) cast doubt on this ability of lichens, current evidence strongly supports the rock weathering activity of these organisms. Biodegradation of silica, silicates, and aluminosilicates is usually a slower process in nature than in the laboratory because the conditions in natural environments are usually less favorable. If this were not so, rock in the biosphere would be a very unstable substance.

Thus, silicon in nature may follow a series of cyclic biogeochemical transformations (Kuznetsov, 1975; Harriss, 1972; Lauwers and Heinen, 1974). Silica, silicates, and aluminosilicates in rocks are subject to the weathering action of biological, chemical, and physical agents. The extent of the contribution of each of these agents must depend on the particular environmental circumstances. Silicon liberated in these processes as soluble silicate may be leached away by surface water or groundwater, and it may be removed from these waters by chemical and/or biological precipitation at new sites, or it may be swept into bodies of freshwater or the sea. There, silicate will tend to be removed by biological agents. Upon their death, these biological agents will release their silicon back into solution or their siliceous remains will be incorporated into the sediment (e.g., Allison, 1981; Patrick and Holding, 1985; Weaver and Wise, 1974), where some or all of the silicon may later be returned to solution by weathering. The sediments of the ocean appear to be a sink for excess silica swept

into the oceans, because the silica concentration of seawater tends to remain relatively constant. But over geologic time, even this silicon is not permanently immobilized. Plate tectonics will ultimately cause even this silicon to be recycled.

9.6 SUMMARY

The environmental distribution of silicon is significantly influenced by microbial activity. Certain microorganisms assimilate it and build it into cell support structures. They include diatoms, some chrysophytes, silicoflagellates, some xanthophytes, radiolarians, and actinopods. Silicon uptake rates by diatoms have been measured, but the mechanism by which silicon is assimilated is still only partially understood. Certain silicon-incorporating bacteria may provide a biochemical model for some aspects of silicon assimilation. Silicon-assimilating microorganisms such as diatoms and radiolaria are important in the formation of siliceous oozes in oceans, and diatoms are important in forming such oozes in lakes.

Some bacteria, fungi, and lichens are able to solubilize silicates and silica. They accomplish this by forming chelators, acids, bases, and/or exopolysaccharides that react with silica and silicates. These reactions are important in weathering of rock and in cycling silicon in nature.

REFERENCES

Adamo P, Violante P. 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. Appl Clay Sci 16:229–256.

Ahmadjian V. 1967. The Lichen Symbiosis. Waltham, MA: Blaidsell.

Allison CW. 1981. Siliceous microfossils from the Lower Cambrian of northwest Canada: Possible source for biogenic chert. Science 211:53–55.

Anderson GM. 1972. Silica solubility. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 1085–1988.

Aristovskaya TV, Kutuzova RS. 1968. Microbiological factors in the mobilization of silicon from poorly soluble natural compounds. Pochvovedenie 12:59–66.

Avakyan ZA, Belkanova NP, Karavaiko GI, Piskunov VP. 1985. Silicon compounds in solution during bacterial quartz degradation. Mikrobiologiya 54:301–307 (Engl transl pp 250–256).

Avakyan ZA, Pivovarova TA, Karavaiko GI. 1986. Properties of a new species, *Bacillus mucilaginosus*. Mikrobiologiya 55:477–482 (Engl transl pp 477–482).

Azam F. 1974. Silicic-acid uptake in diatoms studied with [^{68}Ge]germanic acid as tracer. Planta 121:205–212.

Azam F, Volcani B. 1974. Role of silicon in diatom metabolism. VI. Active transport of germanic acid in the heterotrophic diatom *Nitschia alba*. Arch Microbiol 101:1–8.

Azam F, Hemmingsen B, Volcani BE. 1973. Germanium incorporation into the silica of diatom cell walls. Arch Mikrobiol 92:11–20.

Azam F, Hemmingsen B, Volcani BE. 1974. Role of silicon in diatom metabolism. V. Silicic acid transport and metabolism in the heterotrophic diatom *Nitschia alba*. Arch Microbiol 97:103–114.

Barker WW, Banfield JF. 1996. Biologically versus inorganically mediated weathering reactions: Relationships between minerals and extracellular microbial polymers in lithobionitic communities. Chem Geol 132:55–69.

Barker WW, Banfield JF. 1998. Zones of chemical and physical interactions at interfaces between microbial communities and minerals: A model. Geomicrobiol J 15:223–244.

Barker WW, Welch SA, Banfield JF. 1997. Biogeochemical weathering of silicate minerals. In: Banfield JF, Nealson KH, eds. Geomicrobiology: Interactions Between Microbes and Minerals. Rev Mineralogy, Vol 35. Washington, DC: Mineral Soc Am, pp 391–428.

Bennett PC, Melcer ME, Siegel DI, Hassett JP. 1988. The dissolution of quartz in dilute aqueous solutions of organic acids at 25°C. Geochim Cosmochim Acta 52:1521–1530.

Bennett PC, Hiebert FK, Choi WJ. 1996. Microbial colonization and weathering of silicates in a petroleum-contaminated aquifer. Chem Geol 132:45–53.

Bennett PC, Rogers JR, Choi WJ, Hiebert FK. 2001. Silicates, silicate weathering, and microbial ecology. Geomicrobiol J 18:3–19.

Berner RA, Sjoeberg EL, Velbel MA, Krom MD. 1980. Dissolution of pyroxenes and amphiboles during weathering. Science 207:1205–1206.

Blank GS, Sullivan CW. 1979. Diatom mineralization of silicic acid. III. $Si(OH)_4$ binding and light dependent transport in *Nitschia angularis*. Arch Microbiol 123:157–164.

Bowen HJM. 1966. Trace Elements in Biochemistry. London, UK: Academic Press.

Bowen HJM. 1979. Environmental Chemistry of the Elements. London, UK: Academic Press.

Browne BA, Driscoll CT. 1992. Soluble aluminum silicates: Stoichiometry, stability, and implications for environmental geochemistry. Science 256:1667–1670.

Callot B, Maurette M, Pottier L, Dubois A. 1987. Biogenic etching of microfractures in amorphous and crystalline silicates. Nature (Lond) 328:147–149.

Chisholm SW, Azam F, Epply RW. 1978. Silicic acid incorporation in marine diatoms on light-dark cycles: Use as an assay for phased cell division. Limnol Oceanogr 23:518–529.

Coombs J, Volcani BE. 1968. The biochemistry and fine structure of silica shell formation in diatoms. Silicon induced metabolic transients in *Navicula pelliculosa*. Planta 80:264–279.

Cromack K Jr, Sollins P, Graustein WC, Speidel K, Todd AW, Spycher G, Li CY, Todd RL. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biol Biochem 11:463–468.

Darley WM, Volcani BE. 1969. Role of silicon in diatom metabolism. A silicon requirement for deoxyribonucleic acid synthesis in the diatom *Cylindrotheca fusiformis.* Reiman and Lewin. Exp Cell Res 58:334–343.

Dashman T, Stotzky G. 1986. Microbial utilization of amino acids and a peptide bound on homoionic montmorillonite and kaolinite. Soil Biol Biochem 18:5–14.

De La Rocha CL, Brzezinski MA, DeNiro MJ. 1997. Fractionation of silicon isotopes by marine diatoms during biogenic silica formation. Geochim Cosmochim Acta 61:5051–5056.

De La Rocha CL, Brzezinski MA, DeNiro MJ. 2000. A first look at the distribution of the stable isotopes of silicon in natural waters. Geochim Cosmochim Acta 64:2467–2477.

de Vrind-de Jong EW, de Vrind JPM. 1997. Algal deposition of carbonates and silicates. In: Banfield JF, Nealson KH, eds. Geomicrobiology: Interactions Between Microbes and Minerals. Rev Mineral, Vol 35. Washington, DC: Mineral Soc Am, pp 267–307.

Dommergues Y, Mangenot F. 1970. Écologie Microbienne du Sol. Paris: Masson.

Drever JI. 1994. The effect of land plants on weathering of silicate minerals. Geochim Geophys Acta 58:2325–2332.

Duff RB, Webley DM, Scott RO. 1963. Solubilization of minerals and related materials by 2-ketogluconic acid-producing bacteria. Soil Sci 95:105–114.

Eckhardt FEW. 1980. Microbial degradation of silicates. Release of cations from aluminosilicate minerals by yeast and filamentous fungi. In: Oxley TA, Becker G, Allsopp D, eds. Biodeterioration. Proc 4th Int Biodeterioration Symp, Berlin. London, UK: Pitman, pp 107–116.

Ehrlich HL. 1982. Enhanced removal of Mn^{2+} from seawater by marine sediment and clay minerals in the presence of bacteria. Can J Microbiol 28:1389–1395.

Garrels RM. 1965. Silica: Role in the buffering of natural waters. Science 148:69.

Graustein WC, Cromack K Jr, Sollins P. 1977. Calcium oxalate: Occurrence in soils and effect on nutrient and geochemical cycles. Science 198:1252–1254.

Hale ME Jr. 1967. The Biology of Lichens. London, UK: Edward Arnold.

Hall FR. 1972. Silica cycle. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 1082–1085.

Harriss RC. 1972. Silica–Biogeochemical cycle. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 1080–1082.

Healy FP, Combs J, Volcani BE. 1967. Changes in pigment content of the diatom *Navicula pelliculosa* in silicon starvation synchrony. Arch Mikrobiol 59:131–142.

Heinen W. 1960. Silicium-Stoffwechsel bei Mikroorganismen. I. Mitteilung. Aufnahme von Silicium durch Bakterien. Arch Mikrobiol 37:199–210.

Heinen W. 1962. Silicium-Stoffwechsel bei Mikroorganismen. II. Mitteilung. Beziehung zwischen dem Silicat- und Phosphat-Stoffwechsel bei Bakterien. Arch Mikrobiol 41:229–246.

Heinen W. 1963a. Silicium-Stoffwechsel bei Mikroorganismen. III. Mitteilung. Einfluss verschiedener Anionen auf den bakteriellen Si-Stoffwechsel. Arch Mikrobiol 45:145–161.

Heinen W. 1963b. Silicium-Stoffwechsel bei Mikroorganismen. IV. Mitteilung. Die Wirkung organischer Verbindungen, insbesondere Glucose, auf den Silicium-Stoffwechsel bei Bakterien. Arch Mikrobiol 45:162–171.

Heinen W. 1963c. Silicium-Stoffwechsel bei Mikroorganismen. V. Mitteilung. Untersuchungen zur Mobilität der inkorporierten Kieselsäure. Arch Mikrobiol 45:172–178.

Heinen W. 1965. Time-dependent distribution of silicon in intact cells and cell-free extracts of *Proteus mirabilis* as a model of bacterial silicon transport. Arch Biochem Biophys 110:137–178.

Heinen W. 1967. Ion accumulation in bacterial systems. III. Respiration-dependent accumulation of silicate by a particulate fraction from *Proteus mirabilis* cell-free extract. Arch Biochem Biophys 120:101–107.

Heinen W. 1968. The distribution and some properties of accumulated silicate in cell-free bacterial extracts. Acta Bot Neerl 17:105–113.

Heinen W, Lauwers AM. 1988. Leaching of silica and uranium and other quantitative aspects of the lithibiontic colonization in a radioactive thermal spring. Microb Ecol 15:135–149.

Helmcke J-G. 1954. Die Feinstruktur der Kieselsäure und ihre physiologische Bedeutung in Diatomschalen. Naturwissenschaften 11:254–255.

Henderson MEK, Duff RB. 1963. The release of metallic and silicate ions from minerals, rocks and soils by fungal activity. J Soil Sci 14:236–246.

Hiebert FK, Bennett PC. 1992. Microbial control of silicate weathering in organic-rich ground water. Science 258:278–281.

Holzapfel L. 1951. Siliziumverbindungen in biologischen Systemen. Organ. Kieselsäureverbindungen. XX. Miteilung. Z Elektrochem 55:577–580.

Holzapfel L, Engel W. 1954a. über die Abhängigkeit der Wachstumsgeschwindigkeit von *Asperigillus niger* in Kieselsäurelösungen bei O_2-Belüftung. Naturwissenschaften 41:191–192.

Holzapfel L, Engel W. 1954b. Der Einfluss organischer Kieselsäureverbindungen auf das Wachstum von *Aspergillus niger* and *Triticum*. Z Naturforsch 9b:602–606.

Iler R. 1979. Chemistry of Silica. New York: Wiley.

Jones D, Wilson MJ, McHardy WJ. 1981. Lichen weathering of rock-forming minerals: Application of scanning electron microscopy and microprobe analysis. J Microsc 124 (1):95–104.

Jorgensen EG. 1955. Solubility of silica in diatoms. Physiol Plant 8:846–851.

Karavaiko GI, Avakyan ZA, Krutsko VS, Mel'nikova EO, Zhdanov V, Piskunov VP. 1979. Microbiological investigations on a spodumene deposit. Mikrobiologiya 48:502–508 (Engl transl pp 383–398).

Karavaiko GI, Krutsko VS, Mel'nikova EO, Avakyan ZA, Ostroushko YuI. 1980. Role of microorganisms in spodumene degradation. 49:547–551 (Engl transl pp 402–406).

Karavaiko GI, Belkanova NP, Eroshchev-Shak VA, Avakyan ZA. 1984. Role of microorganisms and some physicochemical factors of the medium in quartz destruction. Mikrobiologiya 53:976–981 (Engl transl pp 795–800).

Katami A. 1982. Dissolution rates of silica from diatoms decomposing at various temperatures. Marine Biol (Berlin) 68:91–96.

Kretz R. 1972. Silicon: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 1091–1092.

Kutuzova RS. 1969. Release of silica from minerals as result of microbial activity. Mikrobiologiya 38:714–721 (Engl transl pp 596–602).

Kuznetsov SI. 1975. The role of microorganisms in the formation of lake bottom deposits and their diagenesis. Soil Sci 119:81–88.

Latimer WM, Hildebrand. 1940. Reference Book of Inorganic Chemistry. Rev ed. New York: Macmillan.

Lauwers AM, Heinen W. 1974. Bio-degradation and utilization of silica and quartz. Arch Microbiol 95:67–78.

Lewin JC. 1957. Silicon metabolism in diatoms. IV. Growth and frustule formation in *Navicula pelliculosa*. J Gen Physiol 39:1–10.

Lewin JC. 1965. Silicification. In: Lewin RA, ed. Physiology and Biochemistry of Algae. New York: Academic Press, pp 445–455.

Liebau F. 1985. Structural Chemistry of Silicates. Structure, Bonding and Classification. Berlin, Germany: Springer-Verlag.

Macaskie LE, Empson RM, Cheetham AK, Gey CP, Skarnulis AJ. 1992. Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymatically mediated growth of polycrystalline HUO_2PO_4. Science 257:782–784.

Malinovskaya IM, Kosenko LV, Votselko SK, Podgorskii VS. 1990. Role of *Bacillus mucilaginosus* polysaccharide in degradation of silicate minerals. Mikrobiologiya 59:70–78 (Engl transl pp 49–55).

Marine Chemistry. 1971. A report of the Marine Chemistry Panel of the Committee of Oceanography. Washington, DC: Nat Acad Sci.

Marshall KC. 1971. Sorption interactions between soil particles and microorganisms. In: McLaren AD, Skujins J, eds. Soil Biochemistry, Vol 2. New York: Marcel Dekker, pp 409–445.

Marshman NA, Marshall KC. 1981a. Bacterial growth on proteins in the presence of clay minerals. Soil Biol Biochem 13:127–134.

Marshman NA, Marshall KC. 1981b. Some effect of montmorillonite on the growth of mixed microbial cultures. Soil Biol Biochem 13:135–141.

Maurice PA, Vierkoen MA, Hersman LE, Fulghum JE, Ferryman A. 2001. Enhancement of kaolinite dissolution by an aerobic *Pseudomonas mendocina* bacterium. Geomicrobiol J 18:21–35.

Milde K, Sand W, Wolff W, Bock E. 1983. Thiobacilli of the corroded concrete walls of the Hamburg sewer system. J Gen Microbiol 129:1327–1333.

Millman JD, Boyle E. 1975. Biological uptake of dissolved silica in the Amazon estuary. Science 189:995–997.

Mitchell RL. 1955. Trace elements. In: Bear FE, ed. Chemistry of the Soil. New York: Reinhold, pp 235–285.

Parker CD. 1947. Species of sulfur bacteria associated with corrosion of concrete. Nature (Lond) 159:439.

Patrick S, Holding AJ. 1985. The effect of bacteria on the solubilization of silica in diatom frustules. J Appl Microbiol 59:7–16.

Reeves CD, Volcani BE. 1984. Role of silicon in diatom metabolism. Patterns of protein phosphorylation in *Cylindrotheca fusiformis* during recovery from silicon starvation. Arch Microbiol 137:291–294.

Rogall E. 1939. über den Feinbau der Kieselmembran der Diatomeen. Planta 29:279–291.

Sand W, Bock E. 1984. Concrete corrosion in the Hamburg sewer system. Environ Technol Lett 5:517–528.
Schultze-Lam S, Fortin D, Davis BS, Beveridge TJ. 1996. Mineralization of bacterial surfaces. Chem Geol 132:171–181.
Schwarz K. 1973. A bound form of silicon in glycosaminoglycans and polyuronides. Proc Natl Acad Sci USA 70:1608–1612.
Sillén LG. 1967. The ocean as chemical system. Science 156:1189–1197.
Silverman MP, Munoz EF. 1970. Fungal attack on rock: Solubilization and altered infrared spectra. Science 169:985–987.
Stotzky G. 1986. Influences of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In: Huang PM, Schnitzer M, eds. Interactions of Soil Minerals with Natural Organics and Microbes. SSSA Spec Publ no 17. Madison, WI: Soil Sci Soc Am, pp 305–428.
Sullivan CW. 1971. A silicic acid requirement for DNA polymerase, thymidylate kinase, and DNA synthesis in the marine diatom *Cylindrica fusiformis*. PhD Thesis, Univ Calif.
Sullivan CW, Volcani EB. 1973. Role of silicon in diatom metabolism. The effects of silicic acid on DNA polymerase, TMP kinase and DNA synthesis in *Cyclotheca fusiformis*. Biochem Biophys Acta 308:212–229.
Tan KH. 1986. Degradation of soil minerals by organic acids. In: Huang PM, Schnitzer M, eds. Interactions of Soil Minerals with Natural Organics and Microbes. SSSA Spec Publ 17. Madison, WI: Soil Sci Soc Am, pp 1–27.
Toth SJ. 1955. Colloidal chemistry of soils. In: Bear FE, ed. Chemistry of the Soil. New York: Reinhold, pp 85–106.
Ullman WJ, Kirchman DL, Welch SA, Vandevivere P. 1996. Laboratory evidence for microbially mediated silicate mineral dissolution in nature. Chem Geol 132:11–17.
Vandevivere P, Welch SA, Ullman WJ, Kirchman DL. 1994. Enchanced dissolution of silicate minerals by bacteria at near-neutral pH. Microb Ecol 27:241–251.
Waksman SA, Starkey RL. 1931. The Soil and the Microbe. New York: Wiley.
Weaver FM, Wise SW Jr. 1974. Opaline sediments of the Southeastern Coastal Plain and horizon A: Biogenic origin. Science 184:899–901.
Weaver TL, Dugan PR. 1972. Enhancement of bacterial methane oxidation by clay minerals. Nature (Lond) 237:518.
Webley DM, Duff RB, Mitchell WA. 1960. A plate method for studying the breakdown of synthetic and natural silicates by soil bacteria. Nature (Lond) 188:766–767.
Webley DM, Henderson MEF, Taylor IF. 1963. The microbiology of rocks and weathered stones. J Soil Sci 14:102–112.
Weiss A, Reiff G, Weiss A. 1961. Zur Kenntnis wasserbeständiger Kieselsäureester. Z Anorg Chem 311:151–179.
Welch SA, Ullman WJ. 1993. The effect of organic acids on plagioclase dissolution rates and stoichiometry. Geochim Cosmochim Acta 57:2725–2736.
Welch SA, Vandevivere P. 1995. Effect of microbial and other naturally occurring polymers on mineral dissolution. Geomicrobiol J 12:227–238.
Werner D. 1966. Die Kieselsäure im Stoffwechsel von *Cyclotella cryptica* Reimann, Lewin and Guillard. Arch Mikrobiol 55:278–308.

Werner D. 1967. Hemmung der Chlorophyllsynthese und des $NADP^+$-abhängigen Glyzeraldeyd-3-phosphat-dehydrogenase durch Germaniumsäure bei *Cyclotella cryptica*. Arch Mikrobiol 57:51–60.

10

Geomicrobiology of Aluminum: Microbes and Bauxite

10.1 INTRODUCTION

Aluminum is the most plentiful element in the Earth's crust after silicon and oxygen. Its crustal abundance has been estimated to be 8.19% (Gornitz, 1972; Strahler, 1976, p. 5). Its concentration in rocks and shales amounts to 82,000 ppm, in sandstones 25,000 ppm, and in limestones 4200 ppm. Its average concentration in freshwater has been given as 0.24 ppm and in seawater 0.001 ppm (Bowen, 1966; Marine Chemistry, 1971). The Al concentration in surface seawater may be under biological control according to studies with the diatom *Skeletonema costatum* (Mackenzie et al., 1978; Stoffyn, 1979). However, inorganic control of aluminum in seawater has also been suggested (Hydes, 1979).

Although a metal, aluminum does not occur in a metallic state in nature. This is best explained by the fact that the reduction of aluminum salts or oxides to Al^0 is an extremely endothermic process (ΔG° is $\sim +377\,\text{kcal}\,\text{mol}^{-1}$ Al_2O_3 for the reaction $Al_2O_3 \rightarrow 2Al + 1.5O_2$). Commercial production of aluminum metal involves electrolysis at high temperature of molten Al_2O_3 in cryolite.

Aluminum is a constituent of igneous minerals including feldspars, micas, some pyroxenes and amphiboles, and secondary minerals, especially clays (see

Chap. 9) and the aluminum hydroxides and oxides gibbsite [γ-$Al(OH)_3$], boehmite [γ-AlO(OH)], diaspore [α-AlO(OH)], and corundum (Al_2O_3) (Gornitz, 1972).

Apart from its presence as a constituent of specific minerals, aluminum exists as Al^{3+} in aqueous solution below about pH 5 and mainly as a cationic complex, $Al(OH)_4^-$, above pH 7.4. Between pH 5 and 7.4, the aluminum ion tends to form hydroxyl complexes [$Al(OH)^{2+}$ and $Al(OH)_2^+$] and precipitates as $Al(OH)_3$ at neutral pH (Macdonald and Martin, 1988; Nordstrom and May, 1995; Garcidueñas Piña and Cervantes, 1996). Alumina (Al_2O_3) is more insoluble in water than silica (SiO_2) (Gornitz, 1972). Al^{3+} can be complexed by a variety of organic compounds including hydroxamate siderophores, sugar acids, phenols, phenolic acids, and polyphenols (Vance et al., 1995). It can also form sulfato and phosphato complexes (Nordstrom and May, 1995).

The Al^{3+} ion is toxic to most forms of life because it can react with negatively charged groups on proteins, including enzymes, and with other vital polymers (Harris, 1972; Garcidueñas Piña and Cervantes, 1996). However, a few plants not only tolerate it but may have a requirement for it. The tree *Orites excelsa* contains basic aluminum succinate in its woody tissue. The ashes of the club moss *Lycopodium alponium* (Pteridophyta) can contain as much as 33% Al_2O_3 (see Gornitz, 1972). The bacterium *Pseudomonas fluorescens* can detoxify Al^{3+} in the bulk phase by producing an extracellular phospholipid that sequesters the aluminum (Appanna et al., 1994; Appanna and St. Pierre, 1994; Appanna and Hamel, 1996). The cyanobacterium *Anabaena cylindrica* can detoxify limited amounts of aluminum taken into the cell with the help of intracellularly occurring inorganic polyphosphate granules, which sequester Al^{3+} (Petterson et al., 1985).

A number of bacteria, fungi, and lichens are known to participate in the formation of secondary aluminum-containing minerals through their ability to weather aluminum-containing rock minerals (see Chaps. 4 and 9).

10.2 MICROBIAL ROLE IN BAUXITE FORMATION

The Nature of Bauxite

Bauxite is an ore that contains 45–50% Al_2O_3 in the form of gibbsite, boehmite, and/or diaspore and not more than 20% Fe_2O_3 as hematite, goethite, and/or aluminian goethite. Such ore also contains 3–5% silica and silicates combined (Valeton, 1972). The silicates in the ore are chiefly in the form of kaolinite.

Bauxite is a product of surficial weathering of aluminosilicate minerals in rock (Butty and Chapalaz, 1984). Warm and humid climatic conditions with wet and dry seasons favor the weathering process. The parent material from which bauxite arises may be volcanic and other aluminosilicate rocks, limestone associated with karsts, and alluvium (Butty and Chapalaz, 1984). The weathering

that leads to bauxite formation begins at the surface of an appropriate exposed or buried rock formation and in cracks and fissures. The process includes breakdown of the aluminosilicates in the parent substance, with gradual solubilization of Al, Si, Fe, and other constituents, starting at the mineral surface. The biological contributions to the weathering are favored by warm temperatures and humid conditions (see below). In the case of limestone as parent substance, an important part of the weathering process is the solubilization of the $CaCO_3$. The solubilized products reprecipitate when their concentration and the environmental pH and E_h are favorable. Groundwater flow may transport some of the solubilized constituents away from the site of weathering, contributing to an enrichment of the constituents left behind. The initial stages of weathering produce a material that could serve as precursor of laterite or bauxite. The difference between laterite and bauxite is that the former is richer in iron relative to aluminum, the reverse being true of bauxite. Biotic and abiotic environmental conditions determine whether alumina or bauxite will accumulate (Schellman, 1994).

Bauxite formation in nature is a slow process and impacted by vegetation at the site of formation and by tectonic movement in addition to climate. Vegetation provides cover that protects against erosion of the weathered rocks, limits water evaporation, and may generate weathering agents (Butty and Chapalaz, 1984). It is also the source of nutrients for microbiota that participate in rock weathering and some other aspects of bauxite formation. Tectonic movement contributes to topography and geomorphology in the area of bauxite formation. Alterations in topography as well as variation in climate can affect the groundwater level. Alternating moist and dry conditions are needed during the weathering of host rock for the formation and buildup of the secondary minerals that make up bauxite.

Biological Role in Weathering of the Parent Rock Material

Biological participation in bauxite formation has been suggested in the past. Butty and Chapalaz (1984) invoked microbial activity in controlling pH and E_h. They viewed rock weathering as being promoted by microbes through generation of acids and/or ligands for mobilizing rock components and in direct participation in redox reactions affecting iron, manganese, and sulfur compounds.

A more detailed proposal of biogenic bauxite formation is that of Taylor and Hughes (1975). They concluded that a bauxite deposit on Rennell Island in the South Solomon Sea near Guadalcanal was the result of biodegradation of volcanic ash that originated in eruptions on Guadalcanal, 180 km distant, and was deposited in pockets of karstic limestone, lagoons, on Rennell Island in the Plio-Pleistocene. The authors established that the bauxite deposit, enclosed in dolomitic limestone from a reef, was not derived from residues left after the

dolomite had weathered away. They speculated that sulfate reducers generated CO_2, which caused weathering of aluminosilicates and ferromagnesian minerals in the volcanic ash, giving rise to transient kaolin that would dissolve at low pH to yield Al^{3+} and silicic acid. Bacterial pyrite formation by sulfate reducers would create pH and E_h conditions that favor weathering of the minerals in the volcanic ash. As initial microbial activity subsided owing to nutrient depletion, pH was predicted to rise, resulting in the formation of iron and aluminum oxides, the chief constituents of bauxite. The authors speculated that bacteria played a role in the formation of a gel of the oxides. Uplift in the northwestern part of the island, groundwater flow, and oxidation of the pyrite were seen to play a role in the maturation of the bauxite.

Natarajan et al. (1997) inferred from the presence of members of the bacterial genera *Thiobacillus*, *Bacillus*, and *Pseudomonas*, in the Jamnagar bauxite mines in Gujarat, India, that these microorganisms are involved in bauxite formation. They based their inference on the known ability of these organisms to weather aluminosilicates, to precipitate oxyhydroxides of iron, to dissolve and transform alkaline metal species, and to form alumina, silica, and calcite minerals. On the same basis, they also implicated the fungi of the genus *Cladosporium*, which they suggest can reduce ferric iron and dissolve aluminosilicates.

On the basis of published discussions of bauxite formation (bauxitization) (e.g., Valeton, 1972; Butty and Chapalaz, 1984), the process can be divided into two stages, which may overlap to some extent. The first stage is the weathering of the parent rock or alluvium that leads to the liberation of Al, Fe, and Si from primary and secondary minerals that contain aluminum. The second stage consists of the formation of bauxite from the weathering products. Each of the two stages is thought to be aided by microorganisms.

The Weathering Phase

Extensive evidence exists that bacteria, fungi, and lichens have the ability to weather rock minerals (see Chap. 9). The evidence was amassed in laboratory experiments and by in situ observation. The rock weathering resulted from the excretion of corrosive metabolic products by various microbes. These products include inorganic and organic acids, bases, and/or organic ligands. In instances where oxidizable or reducible rock components are present, enzymatic redox reactions may also come into play. Most studies of microbial weathering have involved aerobic bacteria and fungi. However, anaerobic bacteria must also be considered in some cases of weathering. Many of them are actually a better source of corrosive organic acids needed in rock weathering than are aerobic bacteria.

The products of rock weathering may be soluble or insoluble. In the latter case, they may accumulate as secondary minerals. In bauxitization, Al, Si, and Fe will be mobilized, and pH control helps to segregate these products to some extent by affecting their respective solubilities. The pH itself is apt to be under the control of the microorganisms. Vegetative cover over the site of bauxitization is a source of nutrients required by the microorganisms to grow and to form the weathering reagents. Warm temperature enhances microbial growth and activity. Infiltrating surface water (rain) and groundwater help to separate the soluble from the insoluble weathering products derived from the source material, leaving behind a mineral mixture that will include aluminum and iron oxides, silica, and silicates (especially kaolinite, formed secondarily from interaction of Al^{3+} and silicate ions). The mineral conglomerate is *protobauxite*.

The Bauxite Maturation Phase

In this stage, the protobauxite becomes enriched in aluminum oxides (gibbsite, diaspore, and/or boehmite) by selective removal of iron oxides, silica, and silicates. Such enrichment has been shown to occur in laboratory experiments under anaerobic conditions (Ehrlich et al., 1995; Ehrlich and Wickert, 1997). Unsterilized Australian ore was placed in presterilized columns. The ore was then completely immersed in a sterile sucrose–mineral salts solution. After outgrowth of bacteria resident on the ore had taken place over 3–5 days at 37°C, the columns were fed daily from the bottom with fresh sterile medium over a time interval of 20–30 min, depending on the size of the columns. Control columns in which the outgrowth of bacteria was suppressed by 0.1% or 0.05% thymol added to the nutrient solution fed to these columns were run in parallel. The effluent of spent medium displaced by each addition of fresh medium was collected and analyzed by measuring pH, determining the concentration of solubilized Fe, Si, and Al, and examining the morphology of the bacteria displaced in the effluents.

The content of the columns quickly turned anaerobic as bacteria grew out from the ore. This was indicated by strong foaming and outgassing at the surface of the columns and by detection of significant numbers of Clostridia among the bacteria in the displaced medium in successive effluents from the columns. The evolved gas probably consisted of CO_2 and H_2, which are known to be produced by clostridial fermentation. Analyses of successive effluents showed that the bacteria solubilized iron in the bauxite, which was in the form of hematite, goethite, and/or aluminian goethite, by reducing it to ferrous iron. As expected, solubilization from unground Australian pisolitic bauxite was slower and less extensive than from the same ore preground to a mesh size of −10 (particle size of 2 mm or less). In one experiment with the unground pisolitic bauxite (Ehrlich et al., 1995), 25% of the iron was mobilized in 106 days. In the same time, the bacteria also solubilized 2.2% of the SiO_2/kaolinite in the ore. Al was solubilized

over this time interval to the extent of 5.9%, but whereas Fe and Si were solubilized at a fairly constant rate once the bacteria had grown out from the ore, Al was not solubilized until the pH in the solution in the column had dropped gradually from about 6.5 initially to about 4.5 after about 20 days. At the start of the experiments, the ore contained 50% Al (calculated as Al_2O_3), 20% Fe (calculated as Fe_2O_3), and 6.5% Si (calculated as SiO_2) by weight. No measurable Fe, Si, or Al solubilization took place in the control columns.

Results from column experiments with bauxite samples from different geographical locations (Ehrlich et al., 1995; Ehrlich and Wickert, 1997) support the notion that for optimal aluminum enrichment of protobauxite, the bulk-phase pH should remain above 4.5. In most cases in the field, the pH probably rarely if ever drops below 4.5. This is because bauxite maturation in nature occurs in an open system where the bacterial activity will be much slower and where acidic metabolites are more readily diluted and carried away by moving groundwater than in the column experiments. However, an exception seems to be a deposit in northern Brazil in which bauxite weathering has given rise to a kaolin deposit as a result of iron mobilization (deferritization) and apparently some aluminum mobilization as well (Kotschoubey et al., 1999). The experimental results described above clearly showed that the action of the bacteria that grew from the ore in the columns enriched the ore in aluminum.

The column effluents contained fermentation acids such as acetic and butyric and sometimes neutral solvents such as butanol, acetone or isopropanol, and ethanol, detectable by odor and by spot confirmation using HPLC analysis. With unground pisolitic ore, Fe and Si but not Al solubilization leveled off after about 60 days. With the same ore ground to -2 mm particle size, Fe, Si, and Al solubilization continued at a steady rate over the entire experimental period.

Some pisolites taken from the active and control columns at the end of the experiment with Australian pisolitic ore described above were cross-sectioned, surface polished, and examined microscopically by reflected light and by scanning electron microscopy (SEM) coupled with energy-dispersive X-ray (EDX) analysis. Color images of cross sections of pisolites that had been subjected to bacterial action in the active column showed a distinct bleached rim surrounding a reddish-brown core. Comparable sections of pisolites from the control column showed only a faintly bleached zone surrounding a reddish-brown core. An SEM-EDX image of a cross section of a pisolite that had been acted upon by bacteria showed a significant depletion of iron in the bleached zone around the core, whereas a similar cross section of a pisolite from a control column showed no significant iron depletion. No depletion in Si or Al was visible in either of the cross sections, probably because the percentages removed were too small (see above). Interestingly, cross sections of pisolites collected at the Weipa bauxite deposit in Queensland, Australia (Rintoul and Fredericks, 1995), resemble the cross section of the microbially attacked pisolites in the previously

described experiments. The finding of Rintoul and Fredericks supports the idea that what happened to the pisolites in the experimental columns is representative of a natural process.

The iron-depleted, bleached zone around the undepleted core in the cross sections from pisolites acted upon by bacteria presents an enigma. The pisolites are not porous, as has been shown by placing untreated pisolites in boiling water and noting a lack of effervescence originating from the pisolite surface, indicating an absence of air entrapped in pores. Thus, bacteria cannot penetrate the pisolites to effect iron mobilization by Fe(III) reduction below the pisolite surface. It is proposed that the bacteria bring about iron mobilization by enzymatic reduction of Fe(III) because daily replacement of a major portion of the medium in columns, which would dilute any chemical reductant or extracellular Fe(III)-reducing enzyme in the bulk phase, did not significantly change the rate of iron reduction. Reduction of Fe(III) below the pisolite surface must therefore depend on a nonenzymatic redox mechanism. Such a mechanism may involve the chemical reaction of Fe^{2+} produced microbially at the surface by enzymatic reduction of Fe_2O_3 at the surface:

$$2Fe_2O_{3\ \text{surface}} + (CH_2O) + 8H^+ \rightarrow 4Fe^{2+}_{\text{surface}} + CO_2 + 5H_2O \qquad (10.1)$$

This Fe^{2+} reacts somehow with Fe_2O_3 below the surface:

$$2Fe^{2+}_{\text{surface}} + Fe_2O_{3\ \text{interior}} + 6H^+ \rightarrow 2Fe^{3+}_{\text{surface}} + 2Fe^{2+}_{\text{interior}} + 3H_2O \qquad (10.2)$$

Reaction (10.2) is best visualized as involving the conduction of an electron from an Fe^{2+}_{surface} [reaction (10.3a)] to the interior, where it reacts with the Fe(III) of Fe_2O_3 [reaction (10.3b)]:

$$2Fe^{2+}_{\text{surface}} \rightarrow 2Fe^{3+}_{\text{surface}} + 2e \qquad (10.3a)$$

$$Fe_2O_{3\ \text{interior}} + 2e + 6H^+ \rightarrow 2Fe^{2+}_{\text{interior}} + 3H_2O \qquad (10.3b)$$

The Fe^{3+} generated at the pisolite surface in reaction (10.2) is immediately rereduced to Fe^{2+} by the bacteria. The Fe^{2+} generated at the interior of the pisolite escapes to the exterior through passages created by the solubilization of Fe and Si, and later Al, if the pH drops below 4.5 in the interior. Because reaction (10.2) is thermodynamically unfavorable ($\Delta G_r^\circ = +1.99$ kcal or $+8.32$ kJ), it is the rapid, bacterially catalyzed reduction of Fe(III) at the surface of the pisolites [reaction (10.1)] that provides the energy that makes reaction (10.2) possible. If, for instance, H_2 instead of CH_2O were the reductant in reaction (10.1), then the value of ΔG_r° would be -35.56 kcal (-148.6 kJ). If acetate were the electron donor, the value of ΔG_r° would be -26.9 kcal (-112.4 kJ). The conduction of electrons to the interior of the pisolite to reduce $Fe_2O_{3\ \text{interior}}$ to $Fe^{2+}_{\text{interior}}$ may be similar to a reaction of anaerobic microbial reduction of structural iron(III) within a ferruginous smectite by *Shewanella putrefaciens* MR-1 with formate or lactate

as electron donor (Kostka et al., 1996). Stucki et al. (1987) previously showed that a bacterium indigenous to the clay reduced the structural iron in ferruginous smectites including the smectite used by Kostka et al. (1996). The difference between the reaction with smectites and the one postulated for bauxitic pisolites is that the reduced iron formed in the smectite is not mobilized by the bacteria as is that formed in the pisolites. Structural iron(II) produced in smectite remains in place and can be reoxidized, and thus ferruginous smectite may serve as a renewable terminal electron acceptor (Ernstsen et al., 1998).

Bacterial reduction of hematite in oxisol samples from the Central Plateau of Brazil incubated in the presence of sucrose at 25 °C was reported by Macedo and Bryant (1989). They observed preferential attack of hematite over aluminian goethite. Initial outgrowth of the bacteria from the soil in these experiments required 3–9 weeks. Microbial activity was correlated with a decrease in redness of the soil.

Bacterial Reduction of Fe(III) in Bauxites from Different Locations

Physiologically similar bacterial cultures grew from bauxite from the pisolitic deposit in Australia, from deposits in the Amazon in Brazil, and from the island of Jamaica in the Caribbean Sea (Ehrlich and Wickert, 1997). When the Amazonian and Jamaican ores in columns were fed sucrose–mineral salts medium, behavior similar to that observed with the Australian bauxite was noted with respect to Fe, Si, and Al solubilization and pH changes in successive column effluents. Clostridia were among the bacteria that were first noted in column effluents. The Clostridia from these two ores showed a close phylogenetic relationship to Clostridia from the Australian ore. These similarities suggest that the natural flora associated with the bauxites may play a role in bauxite maturation, i.e., in its enrichment in Al over time. A caveat is, however, that none of the ore samples were collected under controlled conditions that would have prevented contamination of the ore in the collection process or in subsequent storage. On the other hand, the probability that all ore samples were so heavily contaminated during collection and/or subsequent storage that very similar mixed anaerobic bacterial floras would arise after only 3–4 days of incubation in experimental columns seems small.

Other Observations of Bacterial Interaction with Bauxite

Others have demonstrated bacterial interaction with bauxite, mainly for the purpose of testing whether the ore could be made industrially more attractive (biobeneficiation). These interactions occurred generally under aerobic conditions. Anand et al. (1996) found that *Bacillus polymyxa* strain NCIM 2539 was

able to mobilize in shake culture all the calcium and about 45% of the iron from a bauxite ground to 53–74 μm particle size. The bacterial treatment occurred in Bromfield medium containing 2% sucrose at 30 °C. The change in composition of the bauxite was attributed to direct action of the cells and to the action of cellular products such as exopolysaccharides and organic acids. The oxidation state of the mobilized iron was not determined.

Groudev (1987) reported silicon removal by *Bacillus circulans* and *B. mucilaginosus* from low-grade bauxites. The Si mobilization was attributed to the action of exopolysaccharides that were elaborated by the bacteria. Some Al was also mobilized in these experiments.

Ogurtsova et al. (1989) reported variations in the ability of strains of the fungi *Aspergillus niger* and *Penicillium chrysogenum* and various yeasts and pseudomonads to mobilize Al, Fe, and Si from a ground bauxite of which 70% was −74 μm particle size. The oxidation state of the mobilized iron was not reported. The mobilization of Al, Fe, and Si was attributed to the action of metabolic products formed by the test organisms.

In another study, Karavaiko et al. (1989) found that a strain of *Bacillus mucilaginosus* removed Si from bauxite ground to −0.074 mm particle size and incubated in a sucrose–mineral salts medium with a 10% inoculum in shake-flask culture at 30 °C. This Si removal was attributed to the selective adherence of fine particles of ore rich in Si to the exopolysaccharide at the surface of the bacterial cells and not to dissolution. The mycelial fungi *Aspergillus niger* and *A. pullulans*, on the other hand, were able to mobilize varying amounts of Fe, Al, and Si from the same bauxites by dissolution with metabolic acids they produced in a sucrose–mineral salts medium.

Bandyopadhay and Banik (1995) were able to mobilize 39.9% silica and 46.4% iron from a bauxite ore with a mutated strain of *Aspergillus niger* in a laboratory experiment in which the fungus was allowed to grow at the surface of 80 mL of culture liquid at an initial pH of 4.0 in a flask at 30 °C. The culture medium contained glucose as the energy source and $NaNO_3$ as the nitrogen source. The ore was ground to a mesh size of −170 to −200 and then added to the medium at a concentration of 0.3%. The mobilization of the Si and Fe was attributed to action of the organic acids, probably citric and oxalic, produced by the fungus.

10.3 SUMMARY

Aluminum is the third most abundant element in the Earth's crust, silicon and oxygen being more abundant. Of these three elements, aluminum is the only one for which a physiological function has not been found, although a very small number of higher organisms are known to accumulate it. Al^{3+} is generally toxic.

At least one known cyanobacterium and a strain of *E. coli* have each developed a different mechanism of resistance to it. Various microbes are known to participate through weathering action in the formation of some aluminum-containing minerals.

The formation of bauxite (bauxitization), whose major constituents are Al_2O_3 in the form of gibbsite, boehmite, and/or diaspore; Fe_2O_3 in the form of hematite, goethite, and/or aluminian goethite; and SiO_2/aluminosilicate in the form of silica/kaolinite, can be visualized as involving two stages that may overlap to some extent. Evidence to date suggests that microbes are involved in both stages. The source material in bauxitization may be volcanic and other aluminosilicate rocks, limestone associated with karsts, and alluvium. The first stage of bauxitization involves weathering of source rock and the formation of protobauxite, and the second stage the maturation of protobauxite to bauxite. The first stage, if aerobic, may be promoted by bacteria and fungi, and if anaerobic, by facultative and anaerobic bacteria. The second stage is promoted by iron-reducing and fermentative bacteria under anaerobic conditions. The first stage involves the mobilization of Al, Fe, and Si from host rock and the subsequent precipitation of these rock constituents as oxides, silica, and silicate minerals. The second stage involves the selective mobilization of iron oxides and silica/silicate, enriching the solid residue in aluminum. The process is favored in warm, humid climates with alternating wet and dry seasons. The site of formation must be associated with vegetation that can serve as a source of nutrients to the microorganisms and may yield weathering agents as a result of microbial attack of plant residues. In situ, microbes are expected to play a significant role in the control of pH during bauxite maturation to ensure that little of the aluminum oxide is mobilized.

REFERENCES

Anand P, Modak JM, Natarajan KA. 1996. Biobeneficiation of bauxite using *Bacillus polymyxa*: Calcium and iron removal. Int J Mineral Process 48:51–60.

Appanna VD, St Pierre M. 1994. Influence of phosphate on aluminum tolerance in *Pseudomonas fluorescens*. FEMS Microbiol Lett 124:327–332.

Appanna VD, Hamel R. 1996. Aluminum detoxification mechanism in *Pseudomonas fluorescens* is dependent on iron. FEMS Microbiol Lett 143:223–228.

Appanna VD, Kepes M, Rochon P. 1994. Aluminum tolerance in *Pseudomonas fluorescens* ATCC 13525; involvement of a gelatinous lipid-rich residue. FEMS Microbiol Lett 119:295–302.

Bandyopadhay N, Banik AK. 1995. Optimization of physical factors for bioleaching of silica and iron from bauxite ore by a mutant strain of *Aspergillus niger*. Res Ind 40:14–17.

Bowen HJM. 1966. Trace Elements in Biochemistry. London, UK: Academic Press.

Butty DL, Chapalaz CA. 1984. Bauxite genesis. In: Jacob LE Jr, ed. Bauxite. Proc 1984 Bauxite Symp, Los Angeles, CA. New York: Soc Mining Eng Am Inst Mining, Metall, Petrol Eng, pp 111–151.

Ehrlich HL, Wickert LM. 1997. Bacterial action on bauxites in columns fed with full-strength and dilute sucrose-mineral salts medium. In: Lortie L, Bédard P, Gould WD, eds. Biotechnology and the Mining Environment. SP 97-1. Ottawa, Canada: CANMET Natural Resources Canada, pp 74–89.

Ehrlich HL, Wickert LM, Noteboom D, Doucet J. 1995. Weathering of pisolitic bauxite by heterotrophic bacteria. In: Vargas T, Jerez CA, Wiertz JV, Toledo H, eds. Biohydrometallurgical Processing, Vol I. Santiago: Univ Chile, pp 395–403.

Ernstsen V, Gates WP, Stucki JW. 1998. Microbial reduction of structural iron in clays: A renewable resource of reduction capacity. J Environ Qual 27:761–766.

Garcidueñas Piña R, Cervantes C. 1996. Microbial interactions with aluminum. BioMetals 9:311–316.

Gornitz V. 1972. Aluminum: Element and geochemistry. In: Fairbridge RW, ed. Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 21–23.

Groudev S. 1987. Use of heterotrophic microorganisms in mineral biotechnology. Acta Biotechnol 7:299–306.

Harris SA. 1972. Aluminum toxicity. In: Fairbridge RW, ed. Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 27–29.

Hydes DJ. 1979. Aluminum in seawater: Control by inorganic processes. Science 205:1260–1262.

Karavaiko GI, Avakyan ZA, Ogurtsova LV, Safonova OF. 1989. Microbiological processing of bauxite. In: Salley J, McCready RGL, Wichlacz PL, eds. Biohydrometallurgy. Proc Int Symp, Jackson Hole, WY. CANMET SP89-10. Ottawa, Canada: CANMET, Canada Centre for Mineral and Energy Technology, pp 93–102.

Kostka JE, Stucki JW, Nealson KH, Wu J. 1996. Reduction of structural Fe(III) in smectite by a pure culture of *Shewanella putrefaciens* strain MR-1. Clays Clay Min 44:522–529.

Kotschoubey B, De Souza Duarte AL, Truckenbrodt W. 1999. The bauxitic mantle and origin of the kaolin deposit of Morro do Felipe, lower course of the Jari River, Amapa State, northern Brazil. Rev Bras Geocienc 29:331–338.

Macdonald TL, Martin RB. 1988. Aluminum ion in biological systems. Trends Biochem Sci 13:15–19.

Macedo J, Bryant RB. 1989. Preferential microbial reduction of hematite over goethite in a Brazilian oxisol. Soil Sci Soc Am J 53:1114–1118.

Mackenzie FT, Stoffyn M, Wollast R. 1978. Aluminum in seawater: Control by biological activity. Science 199:680–682.

Marine Chemistry. 1971. Report of Marine Chemistry Panel of the Committee of Oceanography. Washington, DC: Nat Acad Sci.

Natarajan KA, Modak JM, Anand P. 1997. Some microbiological aspects of bauxite mineralization and beneficiation. Min Metall Process 14:47–53.

Nordstrom DK, May HM. 1995. Aqueous equilibrium data for mononuclear aluminum species. In: Esposito G, ed. The Environmental Chemistry of Aluminum. Boca Raton, FL: CRC Lewis, pp 39–80.

Ogurtsova LV, Karavaiko GI, Avakyan ZA, Korenevskii AA. 1989. Activity of various microorganisms in extracting elements from bauxite. Mikrobiologiya 58:956–962 (Engl transl pp 774–780).

Petterson A, Kunst L, Berman B, Roomans GM. 1985. Accumulation of aluminum by *Anabaena cylindrica* into polyphosphate granules and cell walls: An X-ray energy-dispersive microanalysis study. J Gen Microbiol 131:2545–2548.

Rintoul L, Fredericks PM. 1995. Infrared microspectroscopy of bauxite pisoliths. Appl Spectrosc 49:1608–1616.

Schellman W. 1994. Geochemical differentiation in laterite and bauxite formation. Catena 21:131–143.

Stoffyn M. 1979. Biological control of dissolved aluminum in seawater: Experimental evidence. Science 203:651–653.

Strahler AN. 1976. Principles of Earth Science. New York: Harper and Row.

Stucki JW, Komadel P, Wilkinson HT. 1987. Microbial reduction of structural iron(III) in smectites. Soil Sci Soc Am J 51:1663–1665.

Taylor GR, Hughes GW. 1975. Biogenesis of the Rennell bauxite. Econ Geol 70:542–546.

Valeton I. 1972. Bauxites. Amsterdam: Elsevier.

Vance GF, Stevenson FJ, Sikora FJ. 1995. Environmental chemistry of aluminum-organic complexes. In: Esposito G, ed. The Environmental Chemistry of Aluminum. Boca Raton, FL: CRC Lewis, pp 169–220.

11

Geomicrobial Interactions with Phosphorus

11.1 BIOLOGICAL IMPORTANCE OF PHOSPHORUS

Phosphorus is an element fundamental to life, being a structural and functional component of all organisms. It is found universally in such vital cell constituents as nucleic acids, nucleotides, phosphoproteins, and phospholipids. It occurs in teichoic and teichuronic acids in gram-positive bacteria, and in phytins (also known as inositol phosphates) in plants. In many types of bacteria and some yeasts it may also be present intracellularly as polyphosphate granules. Simple phosphates can form anhydrides with other phosphates, as in organic and inorganic pyrophosphates (see Fig. 6.2) and polyphosphate. Phosphate is also capable of forming anhydrides with organic acids and amines and with sulfate (as in adenosine phosphosulfate). The phosphate anhydride bond serves to store biochemically useful energy. For example, a free energy change (ΔG°) of -7.3 kcal (-30.6 kJ) per mole of adenosine 5′-triphosphate (ATP) is associated with the hydrolysis of its terminal anhydride bond, yielding adenosine 5′-diphosphate (ADP)+P_i. Unlike many anhydrides, some of those involving phosphate, such as ATP, are unusually resistant to hydrolysis in the aqueous environment (Westheimer, 1987). Chemical hydrolysis of these bonds requires 7 min of

heating in dilute acid (e.g., 1 N HCl) at the temperature of boiling water (Lehninger, 1970, p. 290). At more neutral pH and physiological temperature, hydrolysis proceeds at an optimal rate only in the presence of appropriate enzymes (e.g., ATPase). The relative resistance of phosphate anhydride bonds to hydrolysis is attributable to the negative charges on the phosphates at neutral pH (Westheimer, 1987). It is the probable reason why ATP came to be selected in the evolution of life as a repository and universal transfer agent of chemical energy in biological systems.

11.2 OCCURRENCE IN THE EARTH'S CRUST

Phosphorus is found in all parts of the biosphere. Its gross abundance on the surface of the Earth has been cited by Fuller (1972) to be 0.10–0.12% (wt/wt). It occurs mostly in the form of inorganic phosphates and organic phosphate derivatives. The organic derivatives in soil are mostly phytins (Paul and Clark, 1996). Total phosphorus concentrations in mineral soil range from 35 to 5300 mg kg^{-1} (average 800 mg kg^{-1}) (Bowen, 1979). An average concentration in freshwater is 0.02 mg kg^{-1} (Bowen, 1979) and in seawater 0.09 mg L^{-1} (Marine Chemistry, 1971). The ratio of organic to inorganic phosphorus (P_{org}/P_i) varies widely in these environments. In mineral soil, P_{org}/P_i may range from 1 : 1 to 2 : 1 (Cosgrove, 1967, 1977). In lake water, as much as 50% of the organic fraction may be phytin (i.e., hydrolyzable by phytase) (Herbes et al., 1975). The organic phosphorus in lake water may constitute 80–99% of the total soluble phosphorus. In the particular examples cited by Herbes et al., the total organic phosphorus rarely exceeded 40 μg phosphate per liter. They speculated that phosphatase-hydrolyzable compounds were largely absent because they are much more labile than phytins. Readily measurable phosphatase activity was detected in Sagima and Suruga Bays, Tokyo, by Kobori and Taga (1979) and Taga and Kobori (1978).

11.3 CONVERSION OF ORGANIC INTO INORGANIC PHOSPHORUS AND THE SYNTHESIS OF PHOSPHATE ESTERS

An important source of free organic phosphorus compounds in the biosphere is the breakdown of animal and vegetable matter. On the other hand, living microbes such as *Escherichia coli* and organisms from activated sludge have been found to excrete aerobically assimilated phosphorus as inorganic phosphate when incubated anaerobically (Shapiro, 1967). Organically bound phosphorus is for the most part not directly available to living organisms because it cannot be taken into the cell in this form. To be taken up, it must first be freed from organic

combination through mineralization. This is accomplished through hydrolytic cleavage catalyzed by phosphatases. In soil as much as 70–80% of the microbial population may be able to participate in this process (Dommergues and Mangenot, 1970, p. 266). Active organisms include bacteria such as *Bacillus megaterium*, *B. subtilis*, *B. malabarensis*, *Serratia* sp., *Proteus* spp., *Arthrobacter* spp., *Streptomyces* spp., and fungi such as *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Cunninghamella* sp. (Dommergues and Mangenot, 1970, p. 266; see also Paul and Clark, 1996). These organisms secrete, or liberate upon their death, phosphatases with greater or lesser substrate specificity (Skujins, 1967). Such activity has also been noted in the marine environment (Ayyakkannu and Chandramohan, 1971).

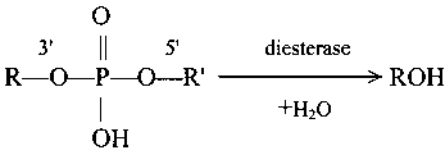
Phosphate liberation from phytin generally requires the enzyme phytase:

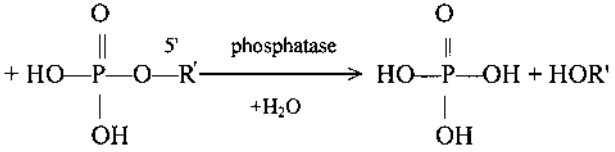
$$\text{Phytin} + 6H_2O \rightarrow \text{inositol} + 6P_i \tag{11.1}$$

Phosphate liberation from nucleic acid requires the action of nucleases, which yield nucleotides, followed by the action of nucleotidases, which yield nucleosides and inorganic phosphate:

$$\text{Nucleic acid} \xrightarrow[+H_2O]{\text{nucleases}} \text{nucleotides} \xrightarrow[+H_2O]{\text{nucleotidase}} \text{nucleoside} + P_i \tag{11.2}$$

Phosphate liberation from phosphoproteins, phospholipids, ribitol, and glycerol phosphates requires phosphomono- and phosphodiesterases. The phosphodiesterases attack phosphodiesters at either the 3′ or 5′ carbon linkage, whereas phosphomonoesterases (phosphatases) attack monoester linkages (Lehninger, 1975, pp. 184, 323–325), e.g.,





(11.3)

Synthesis of organic phosphates (monomeric phosphate esters) is an intracellular process and normally proceeds through a reaction between a carbinol group and ATP in the presence of an appropriate kinase. For example,

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{glucokinase}} \text{glucose 6-phosphate} + \text{ADP} \tag{11.4}$$

Phosphate esters in cells may also arise through phosphorolysis of certain polymers, such as starch or glycogen:

$$(\text{Glucose})_n + \text{H}_3\text{PO}_4 \xrightarrow{\text{phosphorylase}} (\text{glucose})_{n-1} + \text{glucose 1-phosphate} \tag{11.5}$$

$$\text{Glucose 1-phosphate} \xrightarrow{\text{phosphoglucomutase}} \text{glucose 6-phosphate} \tag{11.6}$$

11.4 ASSIMILATION OF PHOSPHORUS

Adenosine 5′-triphosphate (ATP) may be generated from ADP by adenylate kinase,

$$2\text{ADP} \rightarrow \text{ATP} + \text{AMP} \tag{11.7}$$

or by substrate-level phosphorylation, as in the reaction sequence

$$\text{3-Phosphoglyceraldehyde} + \text{NAD}^+ + \text{P}_\text{i} \xrightarrow[\text{dehydrogenase}]{\text{triosephosphate}} \text{1, 3-diphosphoglycerate} + \text{NADH} + \text{H}^+ \tag{11.8}$$

$$\text{1, 3-Diphosphoglycerate} + \text{ADP} \xrightarrow{\text{ADPkinase}} \text{3-phosphoglycerate} + \text{ATP} \tag{11.9}$$

It may also be generated by oxidative phosphorylation:

$$\text{ADP} + \text{P}_\text{i} \xrightarrow[\text{ATPase}]{\text{electron transport system}} \text{ATP} \tag{11.10}$$

or by photophosphorylation:

$$\text{ADP} + \text{P}_\text{i} \xrightarrow[\text{light, ATPase}]{\text{photosynthetic system}} \text{ATP} \tag{11.11}$$

Phosphate polymers are generally produced through reactions such as

$$(\text{Polynucleotide})_{n-1} + \text{nucleotide triphosphate} \xrightarrow{\text{polymerase}} (\text{polynucleotide})_n + \text{P} \sim \text{P} \tag{11.12}$$

In many organisms pyrophosphate (P∼P) is enzymatically hydrolyzed,

$$P \sim P \xrightarrow{\text{pyrophosphatase}} 2P_i \tag{11.13}$$

However, in a few bacteria, inorganic pyrophosphate (P∼P) has been reported to be able to serve as an energy source (Liu et al., 1982; Varma et al., 1983). Whereas it is easy to understand that this ability can be of great importance for energy conservation for intracellularly formed pyrophosphate in bacteria, it remains to be clarified how important it may be for extracellularly available pyrophosphate in nature. Liu et al. (1982) found gram-positive and gram-negative motile and nonmotile bacteria in pyrophosphate enrichments from freshwater anaerobic environments, which grew at the expense of the pyrophosphate as energy source. Nothing appears to be known about the mechanism of pyrophosphate uptake in these organisms.

Like pyrophosphate, intracellular inorganic polyphosphate granules formed by some microbial cells (e.g., Friedberg and Avigad, 1968) are a form of metaphosphate and can represent an energy storage compound (e.g., van Groenestijn et al., 1987) as well as a phosphate reserve. In the case of the cyanobacterium *Anaboena cylindrica* it may also play a role as detoxifying agent by combining with aluminum ions that are taken into the cell (Pettersen et al., 1985) (see also Chap. 10).

11.5 MICROBIAL SOLUBILIZATION OF PHOSPHATE MINERALS

Inorganic phosphorus may occur in soluble and insoluble forms in nature. The most common inorganic form is orthophosphate (H_3PO_4). As an ionic species, the concentration of phosphate is controlled by its solubility in the presence of an alkaline earth cation such as Ca^{2+} or Mg^{2+} or in the presence of metal cations such as Fe^{2+}, Fe^{3+}, or Al^{3+} at appropriate pH values (see Table 11.1). In seawater, for instance, the soluble phosphate concentration (about 3×10^{-6} M, maximum) is controlled by Ca^{2+} ions (4.1×10^2 mg L^{-1}), which form hydroxyapatites with phosphate in a prevailing pH range of ∼7.9–8.1.

TABLE 11.1 Solubility Products of Some Phosphate Compounds

Compound	K_s	Reference
$CaHPO_4 \cdot 2H_2O$	2.18×10^{-7}	Kardos (1955), p. 185
$Ca_{10}(PO_4)_6(OH)_2$	1.53×10^{-112}	Kardos (1955), p. 188
$Al(OH)_2HPO_4$	2.8×10^{-29}	Kardos (1955), p. 184
$FePO_4$	1.35×10^{-18}	From ΔG of formation

Insoluble phosphate occurs most commonly in the form of apatite [$Ca_5(PO_4)_3$(F, Cl, OH)], in which the (F, Cl, OH) radical may be represented exclusively by F, Cl, or OH or any combination of these. In soil, insoluble phosphate may also occur as an aluminum salt (e.g., variscite, $AlPO_4 \cdot 2H_2O$) or vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$].

Insoluble forms of inorganic phosphorus (calcium, aluminum, and iron phosphates) may be solubilized through microbial action. The mechanism by which the microbes accomplish this solubilization varies. One mechanism may be the production of inorganic or organic acids that attack the insoluble phosphates. A second mechanism may be the production of chelators, such as gluconate and 2-ketogluconate (Duff and Webley, 1959; Banik and Dey, 1983; Babu-Khan et al., 1995) (see also Chap. 9), citrate, oxalate, and lactate. All of these chelators can complex the cationic portion of the insoluble phosphate salts and thus force the dissociation of the salts. A third mechanism may be the reduction of iron and ferric phosphate (strengite) to ferrous iron by dissimilatory iron reduction in the reducing environment of sediment–water systems (Jansson, 1987). A fourth mechanism may be the production of hydrogen sulfide (H_2S), which can react with the iron in iron phosphate and precipitate it as iron sulfide, thereby liberating phosphate, as in the reaction

$$2FePO_4 + 3H_2S \rightarrow 2FeS + 2H_3PO_4 + S^0 \qquad (11.14)$$

Table 11.2 lists some organisms active in phosphate solubilization.

Solubilization of phosphate minerals has been noted directly in soil (Alexander, 1977; Babenko et al., 1984; Chatterjee and Nandi, 1964; Dommergues and Mangenot, 1970; Patrick et al., 1973). Indeed, soil containing significant amounts of immobilized calcium, aluminum, or iron phosphates has been thought to benefit from inoculation with phosphate-mobilizing bacteria (see, e.g., discussion by Dommergues and Mangenot, 1970, p. 262). Important microbial phosphate-solubilizing activity in soil occurs in rhizospheres (Alexander, 1977), probably because root secretions allow phosphate-solubilizing bacteria to generate sufficient acid or ligands to effect dissolution of calcium and other insoluble phosphates. Phosphate-deficient soil may be beneficially fertilized with insoluble inorganic phosphate rather than soluble phosphate salts because the former will be solubilized slowly and thus will be better conserved than soluble phosphate salts, which can be readily leached. Soluble phosphate in soil may consist not only of orthophosphate but also of pyrophosphate (metaphosphate). The latter is readily hydrolyzed by pyrophosphatase, especially in flooded soil (Racz and Savant, 1972).

TABLE 11.2 Some Organisms Active in Phosphate Solubilization

Organism	Mechanism of solubilization	Reference
B. megatherium	H_2S production FeS precipitation	Sperber (1958b); Swaby and Sperber (1958)
Thiobacillus sp.	H_2SO_4 production from sulfur	Lipman and McLean (1916)
Nitrifying bacteria	NH_3 oxidation to HNO_3	Dommergues and Mangenot (1970), p. 263
Pseudomonads, *Arthrobacter*, and *Erwinia*-like bacterium	Chelate production; glucose converted to gluconate or 2-ketogluconate	Duff et al. (1963); Sperber (1958a); Dommergues and Mangenot (1970), p. 262; Babu-Khan et al. (1995)
Sclerotium	?	Dommergues and Mangenot (1970), p. 262
A. niger, *A. flavus*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Cylindrosporium* sp., and *Penicillium* sp.	Organic acid production (e.g., citric acid)	Agnihotri (1970)

11.6 MICROBIAL PHOSPHATE IMMOBILIZATION

Microorganisms can cause fixation or immobilization of phosphate, either by promoting the formation of inorganic precipitates or by assimilating the phosphate into organic cell constituents or intracellular polyphosphate granules. The latter two processes have been called transitory immobilization by Dommergues and Mangenot (1970) because of the ready solubilization through mineralization upon the death of the cell. In soil and freshwater environments, transitory immobilization is often more important, although fixation of phosphate by Ca^{2+}, Al^{3+}, and Fe^{3+} is recognized. In a few marine environments (coastal waters or shallow seas) where phosphorite deposits occur, the precipitation mechanism may be more important (McConnell, 1965).

Phosphorite Deposition

Phosphorite (Fig. 11.1) in nature may form authigenically or diagenetically. In the first case, the phosphorite forms as a result of a reaction of soluble phosphate with calcium ions forming corresponding insoluble calcium phosphate compounds. In diagenesis, phosphate may replace carbonate in calcareous concretions. The role of microbes in these processes may be one or more of the following: (1) making reactive phosphate available, (2) making reactive calcium available, or (3)

FIG. 11.1 Micronodules of phosphorite (phosphatic pellets) from the Peru shelf. The average diameter of such pellets is 0.25 mm. According to Burnett (personal communication), such pellets are more representative of what is found in the geologic record than the larger phosphorite nodules. (Courtesy of William C. Burnett.)

generating or maintaining the pH and redox conditions that favor phosphate precipitation.

Authigenic Formations

Models of authigenic phosphorite genesis assume the occurrence of mineralization of organic phosphorus in biologically productive waters, such as at ocean margins, i.e., at shallow depths on continental slopes, on shelf areas, or on plateaus. Here detrital accumulations may be mineralized at the sediment/water interface and in interstitial pore waters, liberating phosphate, some of which may then interact chemically with calcium in seawater to form phosphorite grains. These may subsequently be redistributed within the sediment units (e.g., Riggs, 1984; Mullins and Rasch, 1985). Dissolution of fish debris (bones) has also been considered an important source of phosphate in authigenic phosphorite genesis (Suess, 1981). Upwelling probably plays an important role in many cases of authigenic phosphorite formation on western continental margins at latitudes in both the northern and southern hemispheres, where prevailing winds (e.g., trade winds) cause upwelling (see, e.g., discussion by Burnett et al., 1982; Jahnke et al., 1983; Riggs, 1984). Nathan et al. (1993) cite evidence that in the southern Benguela upwelling system (Cape Peninsula, western coast of South Africa) during non-upwelling periods in winter, phosphate-sequestering bacteria of the oxidative genera *Pseudomonas* and *Acinetobacter* become dominant in the water column. Fermentative Vibrios and Enterobacteriacea are dominant during upwelling in summer. It has been suggested that *Pseudomonas* and *Acinetobacter*, which sequester phosphate as polyphosphate under aerobic conditions and hydrolyze the polyphosphate under anaerobic conditions to obtain energy of maintenance and to sequester volatile fatty acids for polyhydroxybutyrate formation, contribute to authigenic phosphorite formation. Locally elevated, excreted orthophosphate becomes available for precipitation as phosphorite by reacting with seawater calcium. In the northern Benguela upwelling system off the coast of Namibia, where upwelling occurs year-round, Nathan et al. (1993) found that phosphate-sequestering cocci occurred in the water column. They suggested that these organisms, like *Pseudomonas* and *Acinetobacter*, may release sequestered phosphate when they reach waters with low oxygen concentration below 10 m water depth and thereby contribute to phosphorite formation.

Authigenic phosphorite at some eastern continental margins, where upwelling, if it occurs at all, is a weak and intermittent process, may have been formed more directly as a result of intracellular bacterial phosphate accumulation, which became transformed into carbonate fluorapatite upon death of the cells and accumulated in sediments in areas where the sedimentation rate was very low (O'Brien and Veeh, 1980; O'Brien et al., 1981). Ruttenberg and Berner (1993) concluded that carbonate fluorapatite accumulations in Long Island Sound and

Mississippi Delta sediments are the result of mineralization of organic phosphorus. These accumulations increased as organic phosphorus concentrations decreased with depth. Thus important phosphorus sinks occur in sediments of continental margins outside upwelling regions.

Youssef (1965) proposed that phosphorite could be formed in a marine setting through mineralization of phytoplankton remains that have settled into a depression on the seafloor, leading to localized accumulation of dissolved phosphate. According to him, this phosphate could then precipitate as a result of reaction with calcium in seawater. Piper and Codespoti (1975) proposed that carbonate fluorapatite [$Ca_{10}(PO_4,CO_3)_6F_{2-3}$] precipitation in the marine environment may be dependent on bacterial denitrification in the oxygen minimum layer of the ocean as it intersects with the ocean floor. A loss of nitrogen due to denitrification means lowered biological activity and can lead to excess accumulation of phosphate in this zone. The lower pH (pH 7.4–7.9) in the deeper waters compared to the surface waters keep phosphate dissolved and allow for its transport by upwelling to regions where phosphate precipitation is favored (pH > 8) (Fig. 11.2). This model takes into account the conditions of marine apatite formation described by Gulbrandsen (1969) and helps to explain the occurrence of probable contemporary formation of phosphorite in regions of upwelling such as the continental margin of Peru (Veeh et al., 1973; see also, however, Suess, 1981) and on the continental shelves of southwestern Africa (Baturin, 1973; Baturin et al., 1969). To explain the more extensive ancient phosphorite deposits, a periodic warming of the ocean can be invoked, which would reduce oxygen solubility and favor more intense denitrification in deeper waters, resulting in temporarily lessened biological activity and a consequent increase in dissolved phosphate concentration that would lead to phosphate precipitation (Piper and Codespoti, 1975). Mullins and Rasch (1985) proposed an oxygen-depleted sedimentary environment for biogenic apatite formation along the continental margin of central California during the Miocene. They view oolitic phosphorite as having resulted from organic matter mineralization by sulfate reducers in sediments in which dolomite was concurrently precipitated. The phosphate, according to their model, then tended to precipitate interstitially as phosphorite, in part around bacterial nuclei. Mullins and Rasch (1985) found fossilized bacteria in the phosphorite. O'Brien et al. (1981) had previously reported the discovery of fossilized bacteria in a phosphorite deposit on the East Australian margin.

Diagenetic Formation

Models of phosphorite formation through diagenesis generally assume an exchange of phosphate for carbonate in calcium carbonate accretions that have the form of calcite or aragonite. The role of bacteria in this process is to mobilize

phosphate by mineralizing detrital organic matter. This has been demonstrated in marine and freshwater environments under laboratory conditions (Lucas and Prévot, 1984; Hirschler et al., 1990a, 1990b). Adams and Burkhart (1967) propose that diagenesis of calcite to form apatite explains the origin of some deposits in the North Atlantic. Phosphorite deposits off Baja California and in a core from the eastern Pacific Ocean seem to have formed as a result of partial diagenesis (d'Anglejan, 1967, 1968).

Occurrences of Phosphorite Deposits

Sizable phosphorite deposits are associated with only six brief geological intervals: the Cambrian, Ordovician, Devonian-Mississippian, Permian, Cretaceous, and Cenozoic eras. Because in many instances these phosphorite deposits are associated with black shales and contain uranium in reduced form (Altschuler et al., 1958), they are presumed to have accumulated under reducing conditions. Apatite appears to be forming at the present time in the sediments at the Mexican continental margin (Jahnke et al., 1983) and in the deposits off the coast of Peru (e.g., Burnett et al., 1982; Suess, 1981; Veeh et al., 1973).

Deposition of Other Phosphate Minerals

Microbes can also play a role in the authigenic or diagenetic formation of other phosphate minerals such as vivianite, strengite, and variscite. In these instances the bacteria may contribute orthophosphate to the mineral formation by degrading organic phosphate in detrital matter and/or they may contribute iron or aluminum by mobilizing these metals from other minerals. Authigenic formation of such phosphate minerals is probably most common in soil. A case of diagenetic formation of vivianite from siderite ($FeCO_3$) in the North Atlantic coastal plain has been proposed by Adams and Burkhart (1967). Microbial control of pH and E_h can influence the stability of these phosphate minerals (Patrick et al., 1973; Williams and Patrick, 1971).

Citrobacter sp. has been reported to form metal phosphate precipitates, e.g., cadmium phosphate ($CdHPO_4$) and uranium phosphate (UO_2HPO_4), that encrusted the cells (Macaskie et al., 1987, 1992). The precipitates form as a result of the action of a cell-bound, metal-resistant phosphatase on organophosphates such as glycerol-2-phosphate, liberating orthophosphate (HPO_4^{2-}) that reacts in the immediate surround of the cells with metal cations to form corresponding metal phosphates. The metal phosphates form deposits on the cell surface.

Some microbes, such as certain strains of *Arthrobacter*, *Flavobacterium*, *Listeria*, and *Pseudomonas*, can cause struvite ($MgNH_4PO_4 \cdot 6H_2O$) to form, at least under laboratory conditions (Rivadeneyra et al., 1983, 1992a). A major, but

not necessarily exclusive, microbial contribution to this process appears to be ammonium formation (Rivadeneyra et al., 1992b). Struvite forms in seawater solutions in which the NH_4^+ concentration is 0.01 M when orthophosphate is added at pH 8.0 (Handschuh and Orgel, 1973). The presence of calcium ion in sufficient quantity can suppress struvite formation and promote apatite formation instead (Rivadeneyra et al., 1983). Although struvite formation is probably of little significance in nature today, it may have been significant in the primitive world of Precambrian times if NH_4^+ was present in concentrations as high as 10^{-2} M (Handschuh and Orgel, 1973).

11.7 MICROBIAL REDUCTION OF OXIDIZED FORMS OF PHOSPHORUS

Phosphorus may also undergo redox reactions, some or all of which may be catalyzed by microbes. Of biogenic interest are the +5, +3, +1, and −3 oxidation states, as in orthophosphate (H_3PO_4), orthophosphite (H_3PO_3), hypophosphite (H_3PO_2), and phosphine (PH_3), respectively. Reduction of phosphate to phosphine by soil bacteria has been reported (Rudakov, 1927; Tsubota, 1959; Devai et al., 1988). Mannitol appeared to be a suitable electron donor in the reaction described by Rudakov (1927) and glucose in the experiments described by Tsubota (1959). Phosphite and hypophosphite were claimed to be intermediates in the reduction (Rudakov, 1927; Tsubota, 1959). Devai et al. (1988) detected phosphine evolution in anaerobic sewage treatment in Imhoff tanks in Hungary and confirmed the observation in anaerobic laboratory experiments. Gassmann and Schorn (1993) detected phosphine in surface sediments in Hamburg Harbor. The phosphine was most readily detected in porous sediments, in which the porosity was due to gas bubbles. Iron phosphide (Fe_3P_2) is reported to have been formed when a cell-free preparation of *Desulfovibrio* was incubated in the presence of steel in a yeast extract broth under hydrogen gas (Iverson, 1968). Inositol hexaphosphate, a product of plants and present in yeast extract, may be a substrate for phosphine formation (Iverson, 1998). Hydrogenase from *Desulfovibrio* may have been responsible for the formation of phosphine from the inositol phosphate in the yeast extract using the hydrogen in the system as the reductant in Iverson's (1968) experiment. The phosphine could then have reacted with ferrous iron from the steel corrosion to form Fe_3P_2 (Iverson, 1968).

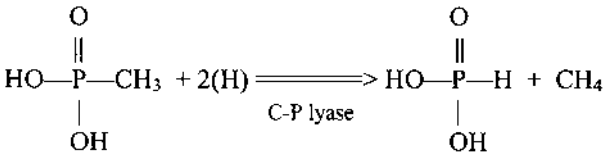
Questions have been raised about the ability of microbes to reduce phosphate. Liebert (1927) showed that on the basis of thermodynamic calculations using heats of formation, the reduction of phosphate to phosphite by mannitol is an energy-consuming process and could therefore not serve a

respiratory function. He calculated a heat of reaction value (ΔH) of +20 kcal on the basis of the following equation:

$$\underset{316\ \text{kcal}}{C_6H_{14}O_6} + 13Na_2HPO_4 \rightarrow \underset{5390\ \text{kcal}}{13Na_2HPO_3} + \underset{4460\ \text{kcal}}{6CO_2} + \underset{566\ \text{kcal}}{7H_2O} + \underset{478\ \text{kcal}}{} \qquad (11.15)$$

He also calculated a ΔH of +438 kcal for the reduction of phosphate to hypophosphite and a ΔH of +1147 kcal for the reduction of phosphate to phosphine. These conclusions can also be reached when free energy changes (ΔG) are considered instead of heats of formation (ΔH). Woolfolk and Whiteley (1962) reported that they were unable to reduce phosphate with hydrogen in the presence of an extract of *Veillonella alcalescens* (formerly *Micrococcus lactilyticus*), even though this extract could catalyze the reduction of some other oxides with hydrogen. Skinner (1968) also questioned the ability of bacteria to reduce phosphate. He could not find such organisms in soils he tested. Burford and Bremner (1972), while unable to demonstrate phosphine evolution from waterlogged soils, were not able to rule out microbial phosphine genesis because they found that soil constituents can adsorb phosphine. Thus, unless bacteria form phosphine in excess of the adsorption capacity of a soil, phosphine detection in the gas phase may not be possible.

Interestingly, Barrenscheen and Beckh-Widmanstetter (1923) reported the production of hydrogen phosphide (phosphine, PH_3) from organically bound phosphate during putrefaction of beef blood. Much more recently, Metcalf and Wanner (1991) presented evidence supporting the existence of a C-P lyase in *Escherichia coli* that catalyzes the reductive cleavage of compounds such as methyl phosphonate to phosphite and methane,



(11.16)

This enzyme activity was previously studied in *Agrobacterium radiobacter* (Wackett et al., 1987), although it was described by these authors as a hydrolytic enzyme. Phosphonolipids are known to exist in organisms from bacteria to mammals (Hilderbrand and Henderson, 1989, cited by Metcalf and Wanner, 1991). Thus biochemical mechanisms for synthesizing organophosphonates exist, and it is therefore highly likely that an organophosphonate such as methyl- or ethylphosphonate that requires a C-P lyase to release the phosphorus (Metcalf and Wanner, 1991) is an intermediate in the conversion of orthophosphate to

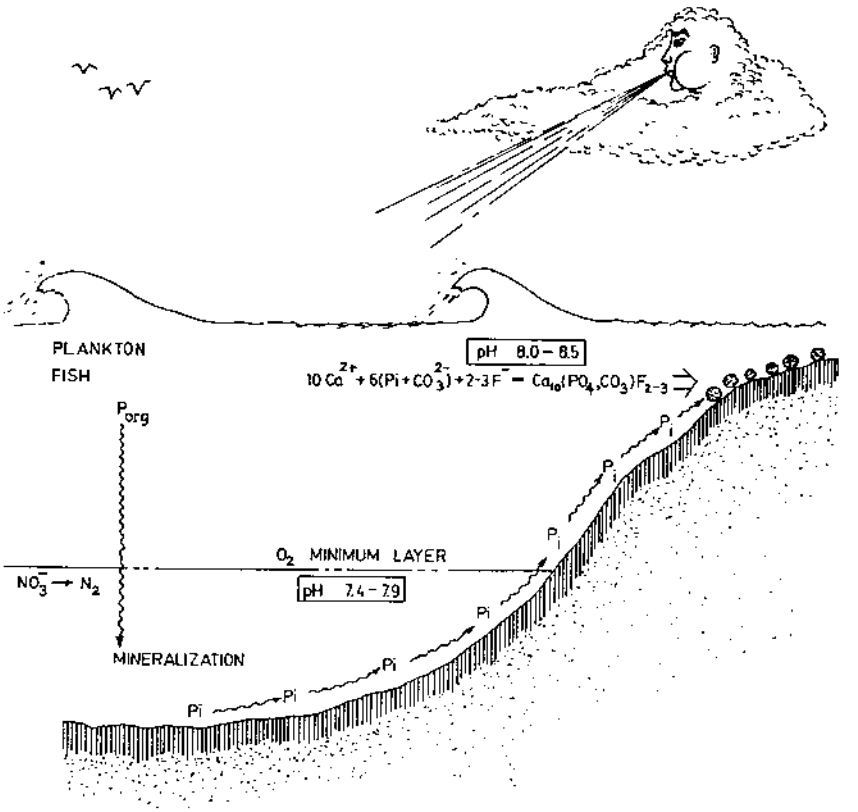


FIG. 11.2 Schematic representation of phosphorite formation in the marine environment according to the model of Piper and Codispoti (1975). Note that the rising P_i upslope is due to upwelling.

orthophosphite and that C-P lyase activity represents the reductive step in this transformation. This needs further investigation.

11.8 MICROBIAL OXIDATION OF REDUCED FORMS OF PHOSPHORUS

Reduced forms of phosphate can be aerobically and anaerobically oxidized by bacteria. Thus *Bacillus caldolyticus*, a moderate thermophile, can oxidize hypophosphite to phosphate aerobically (Heinen and Lauwers, 1974). The

active enzyme system consists of an $(NH_4)_2SO_4$-precipitable protein fraction, NAD, and respiratory chain components. The enzyme system does not oxidize phosphite. Adams and Conrad (1953) first reported the aerobic oxidation of phosphite to phosphate by bacteria and fungi from soil. All phosphite that was oxidized by these strains was assimilated. None of the oxidized phosphite, i.e., phosphate, was released into the medium before the organisms died. Phosphate added to the medium inhibited phosphite oxidation. Active organisms included the bacteria *P. fluorescens*, *P. lachrymans*, *Aerobacter* (now known as *Enterobacter*) *aerogenes*, *Erwinia amylovora*; the fungi *Alterneria*, *Aspergillus niger*, *Chaetomium*, *Pencillium notatum*; and some actinomycetes. In later studies, Casida (1960) found that a culture of *P. fluorescens* strain 195 formed orthophosphate aerobically from orthophosphite in excess of its needs and released phosphate into the medium. The culture was heterotrophic, and its phosphite-oxidizing activity was inducible and stimulated by yeast extract. The enzyme system involved in phosphite oxidation was an orthophosphite-nicotinamide adenine dinucleotide oxidoreductase, which was inactive on arsenite, hypophosphite, nitrite, selenite, or tellurite and was inhibited by sulfite (Malacinski and Konetzka, 1966, 1967).

Oxidation of reduced phosphorus compounds can also occur anaerobically. A soil bacillus has been isolated that is capable of anaerobic oxidation of hypophosphite and phosphite to phosphate (Foster et al., 1978). In a mixture of phosphite and hypophosphite, phosphite was oxidized first. Phosphate inhibited the oxidation of either phosphite or hypophosphite. The organism did not release phosphate into the medium.

Because phosphite and hypophosphite have not been reported in detectable quantities in natural environments, it has been suggested that microbial ability to utilize the compounds, especially anaerobically, may be a vestigial property developed at a time when the Earth had a reducing atmosphere surrounding it that favored the occurrence of phosphite (Foster et al., 1978).

11.9 MICROBIAL ROLE IN THE PHOSPHORUS CYCLE

In many ecosystems, phosphorus availability may determine the extent of microbial growth and activity. The element follows cycles in which it finds itself alternately outside and inside living cells, in organic and inorganic form, free or fixed, dissolved or precipitated. Microbes play a central role in these changes of state, as outlined in Figure 11.3 and as discussed in this chapter.

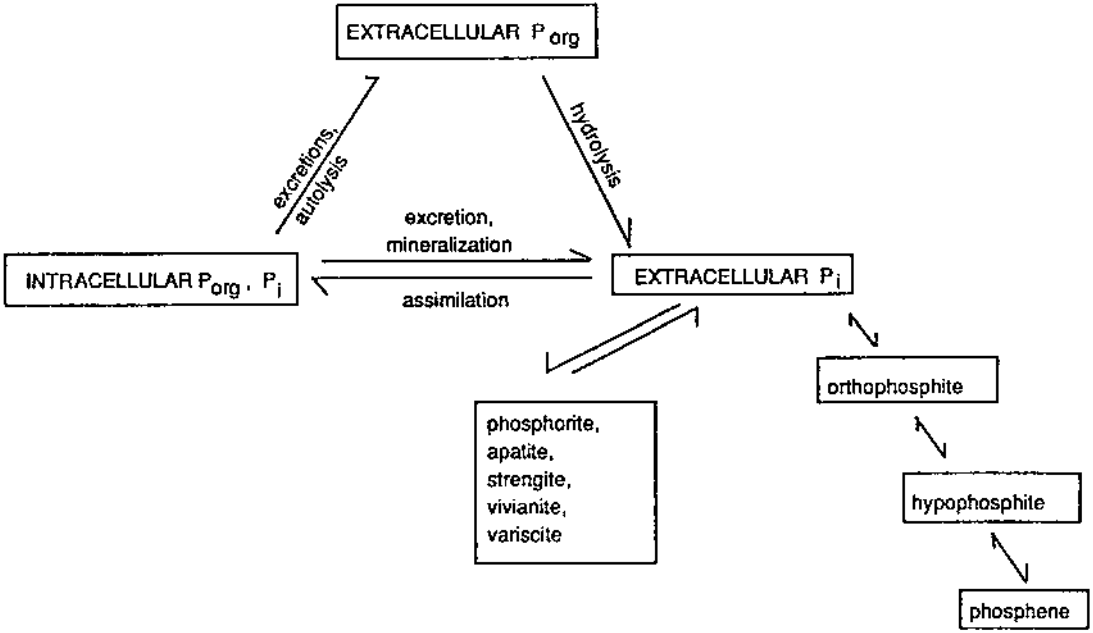


FIG. 11.3 The phosphorus cycle.

11.10 SUMMARY

Phosphorus is a very important element for all forms of life. It is used in cell structure as well as cell function. It plays a role in transducing biochemically useful energy. When free in the environment, it occurs primarily as organic phosphate esters and as inorganic phosphates. Some of the latter, such as calcium, aluminum, and iron phosphates, are very insoluble at neutral or alkaline pH. To be nutritionally available, organic phosphates have to be enzymatically hydrolyzed to liberate orthophosphate. Microbes play a central role in this process. Microbes may also free orthophosphate from insoluble inorganic phosphates by producing organic or mineral acids or chelators, or, in the case of iron phosphates, by producing H_2S. Under some conditions, microbes may promote the formation of insoluble inorganic phosphates, such as those of calcium, aluminum, or iron. They have been implicated in phosphorite formation in the marine environment.

Microbes have been implicated in the reduction of pentavalent phosphorus to lower valence states. The experimental evidence for this is somewhat equivocal, however. It is likely that organic phosphorus compounds are intermediates in these reductions. Microbes have also been implicated in the aerobic and anaerobic oxidation of reduced forms of phosphorus to phosphate. The experimental evidence in this case is strong. It includes demonstration of enzymatic involvement. The geomicrobial significance of these redox reactions in nature is not clearly understood.

REFERENCES

Adams JK, Burkhart B. 1967. Diagenetic phosphates from the northern Atlantic coastal plain. Abstr Annu Geophys Soc Am Assoc Soc Joint Meeting, New Orleans, LA. Nov 20–22, 1967. Program, p 2.

Adams F, Conrad JP. 1953. Transition of phosphite to phosphate in soil. Soil Sci 75:361–371.

Agnihotri VP. 1970. Solubilization of insoluble phosphates by some soil fungi isolated from nursery seedbeds. Can J Microbiol 16:877–880.

Alexander M. 1977. Introduction to Soil Microbiology. 2nd ed. New York: Wiley.

Altschuler S, Clarke RS Jr, Young EJ. 1958. Geochemistry of uranium in apatite and phosphorite. US Geol Surv Prof Pap 314D.

Ayyakkannu K, Chandramohan D. 1971. Occurrence and distribution of phosphate solubilizing bacteria and phosphatase in marine sediments at Porto Nova. Mar Biol 11:201–205.

Babenko YuS, Tyrygina GI, Grigor'ev EF, Dolgikh LM, Borisova TI. 1984. Biological activity and physiological-biochemical properties of phosphate dissolving bacteria. Mikrobiologiya 53:533–539 (Engl transl pp 427–433).

Babu-Khan S, Yeo TC, Martin WL, Duran MR, Rogers RD, Goldstein AH. 1995. Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas capacia*. Appl Environ Microbiol 61:972–978.

Banik S, Dey BK. 1983. Phosphate solubilizing potentiality of the microorganisms capable of utilizing aluminum phosphate as a sole phosphate source. Z Mikrobiol 138:17–23.

Barrenscheen HK, Beckh-Widmanstetter HA. 1923. Über bakterielle Reduktion organisch gebundener Phosphorsäure. Biochem Z 140:279–283.

Baturin GN. 1973. Genesis of phosphorites of the Southwest African shelf. Tr Inst Okeanol Akad Nauk SSSR 95:262–267.

Baturin GN, Merkhulova KI, Chalov PI. 1969. Radiometric evidence for recent formation of phosphatic nodules in marine shelf sediments. Mar Geol 13:M37–M41.

Bowen HJM. 1979. Environmental Chemistry of the Elements. London: Academic Press.

Burford JR, Bremner JM. 1972. Is phosphate reduced to phosphine in waterlogged soils? Soil Biol Biochem 4:489–495.

Burnett WC, Beers MJ, Roe KK. 1982. Growth rates of phosphate nodules from the continental margin off Peru. Science 215:1616–1618.

Casida LE Jr. 1960. Microbial oxidation and utilization of orthophosphite during growth. J Bacteriol 80:237–241.

Chatterjee AK, Nandi P. 1964. Solubilization of insoluble phosphates by rhizosphere fungi of legumes (*Arachis cyanopsis*, *Desmodium*). Trans Bose Res Inst (Calcutta) 27:115–120.

Cosgrove DJ. 1967. Metabolism of organic phosphates in soil. In: McLaren AD, Petersen GH, eds. Soil Biochemistry, Vol 1. New York: Marcel Dekker, pp 216–228.

Cosgrove DJ. 1977. Microbial transformations in the phosphorus cycle. Adv Microb Ecol 1:95–134.

d'Anglejan BF. 1967. Origin of marine phosphates off Baja California, Mexico. Mar Geol 5:15–44.

d'Anglejan BF. 1968. Phosphate diagenesis of carbonate sediments as a mode of in situ formation of marine phosphorites: Observations in a core from the Eastern Pacific. Can J Earth Sci 5:81–87.

Devai I, Feldoeldy L, Wittner I, Plosz S. 1988. Detection of phosphine: New aspects of the phosphorus cycle in the hydrosphere. Nature (Lond) 333:343–345.

Dommergues Y, Mangenot F. 1970. Écologie Microbienne du Sol. Paris: Masson.

Duff RB, Webley DM. 1959. 2-Ketogluconic acid as a natural chelator produced by soil bacteria. Chem Ind pp 1376–1377.

Duff RB, Webley DM, Scott RO. 1963. Solubilization of minerals and related materials by 2-ketogluconic acid-producing bacteria. Soil Sci 95:105–114.

Foster TL, Winans L Jr, Helms JS. 1978. Anaerobic utilization of phosphite and hypophosphite by *Bacillus* sp. Appl Environ Microbiol 35:937–944.

Friedberg I, Avigad G, 1968. Structures containing polyphosphate in *Micrococcus lysodeikticus*. J Bacteriol 96:544–553.

Fuller WH. 1972. Phosphorus: Element and Geochemistry. In: Fairbridge WR, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 942–946.

Gassmann G, Schorn F. 1993. Phosphine from harbor surface sediments. Naturwissenschaften 80:78–80.

Gulbrandsen RA. 1969. Physical and chemical factors in the formation of marine apatite. Econ Geol 64:365–382.

Handschuh GJ, Orgel LE. 1973. Struvite and prebiotic phosphorylation. Science 179:483–484.

Heinen W, Lauwers AM. 1974. Hypophosphite oxidase from *Bacillus caldolyticus*. Arch Microbiol 95:267–274.

Herbes SE, Allen HE, Mancy KH. 1975. Enzymatic characterization of soluble organic phosphorus in lake water. Science 187:432–434.

Hilderbrand RL, Henderson TO. 1989. Phosphonic acids in nature: In: Hilderbrand RL, ed. The Role of Phosphonates in Living Systems. Boca Raton, FL: CRC, pp 6–25.

Hirschler A, Lucas J, Hubert J-C. 1990a. Bacterial involvement in apatite genesis. FEMS Microbiol Ecol 73:211–220.

Hirschler A, Lucas J, Hubert J-C. 1990b. Apatite genesis: A biologically induced or biologically controlled mineral formation process? Geomicrobiol J 7:47–57.

Iverson WP. 1968. Corrosion of iron and formation of iron phosphide by *Desulfovibrio desulfuricans*. Nature (Lond) 217:1265.

Iverson WP. 1998. Possible source of a phosphorus compound produced by sulfate-reducing bacteria that cause anaerobic corrosion of iron. Mater Perform 37:46–49.

Jahnke RA, Emerson SR, Roe KK, Burnett WC. 1983. The present day formation of apatite in Mexican continental margin sediments. Geochim Cosmochim Acta 47:259–266.

Jansson M. 1987. Anaerobic dissolution of iron-phosphorus complexes in sediment due to the activity of nitrate-reducing bacteria. Microb Ecol 14:81–89.

Kardos LT. 1955. Soil fixation of plant nutrients. In: Bear FE, ed. Chemistry of the Soil. New York: Reinhold, pp 177–199.

Kobori H, Taga N. 1979. Phosphatase activity and its role in the mineralization of organic phosphorus in coastal sea water. J Exp Mar Biol Ecol 36:23–39.

Lehninger AL. 1970. Biochemistry. New York: Worth.

Lehninger AL. 1975. Biochemistry. 2nd ed. New York: Worth.

Liebert F. 1927. Reduzieren Mikroben Phosphat? Z Bakt Parasitenk Infektionskr Hyg 72:369–374.

Lipman JG, McLean H. 1916. The oxidation of sulfur soils as a means of increasing the availability of mineral phosphates. Soil Sci 1:533–539.

Liu CL, Hart N, Peck HD Jr. 1982. The utilization of inorganic pyrophosphate as a source of energy for the growth of sulfate reducing bacteria, *Desulfotomaculum* sp. Science 217:363–364.

Lucas J, Prévot L. 1984. Synthèse de l'apatite par voie bactérienne á partir de matière organique phosphatée et de divers carbonates de calcium dans des eaux douce et marine naturelles. Chem Geol 42:101–118.

Macaskie LE, Dean ACR, Cheetham AK, Jakeman RJB, Skarnulis AJ. 1987. Cadmium accumulation by a *Citrobacter* sp.: The chemical nature of the accumulated metal precipitate and its location on the bacterial cells. J Gen Microbiol 133:539–544.

Macaskie LE, Empson RM, Cheetham AK, Grey CP, Skarnulis AJ. 1992. Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymatically mediated growth of polycrystalline HUO_2PO_4. Science 257:782–784.

Malacinski G, Konetzka WA. 1966. Bacterial oxidation of orthophosphite. J Bacteriol 91:578–582.
Malacinski G, Konetzka WA. 1967. Orthophosphite-nicotinamide adenine dinucleotide oxidoreductase from *Pseudomonas fluorescens*. J Bacteriol 93:1906–1910.
Marine Chemistry. 1971. A Report of the Marine Chemistry Panel of the Committee of Oceanography. Washington, DC: Natl Acad Sci.
McConnell D. 1965. Precipitation of phosphates in sea water. Econ Geol 60:1059–1062.
Metcalf WW, Wanner BL. 1991. Involvement of the *Escherichia coli phn* (φD) gene cluster in assimilation of phosphorus in the form of phosphonates, phosphite, P_i esters, and P_i. J Bacteriol 173:587–600.
Mullins HT, Rasch RF. 1985. Sea-floor phosphorites along the Central California continental margin. Econ Geol 80:696–715.
Nathan Y, Bremner JM, Loewenthal RE, Monteiro P. 1993. Role of bacteria in phosphorite genesis. Geomicrobiol J 11:69–76.
O'Brien GWO, Veeh HH. 1980. Holocene phosphorite on the East Australian margin. Nature (Lond) 288:690–692.
O'Brien GWO, Harris JR, Milnes AR, Veeh HH. 1981. Bacterial origin of East Australian continental margin phosphorites. Nature (Lond) 294:442–444.
Paul EA, Clark FE. 1996. *Soil Microbiology and Biochemistry.* 2nd ed. San Diego: Academic Press.
Patrick WH Jr, Gotoh S, Williams BG. 1973. Strengite dissolution in flooded soils and sediment. Science 179:564–565.
Petterson, A, Kunst L, Bergman B, Roomans GM. 1985. Accumulation of aluminum by *Anabaena cylindrica* into polyphosphate granules and cell walls: An X-ray energy dispersive microanalysis study. J Gen Microbiol 1312:2545–2548.
Piper DZ, Codespoti LA. 1975. Marine phosphorite deposits and the nitrogen cycle. Science 179:564–565.
Racz GJ, Savant NK. 1972. Pyrophosphate hydrolysis in soil as influenced by flooding and fixation. Soil Sci Soc Am Proc 36:678–682.
Riggs SR. 1984. Paleooceanographic model of neogene phosphorite deposition, US Atlantic Continental Margin. Science 223:123–131.
Rivadeneyra MA, Ramos-Cormenzana A, Garcia-Cervigon A. 1983. Bacterial formation of struvite. Geomicrobiol J 3:151–163.
Rivadeneyra MA, Perez-Garcia I, Ramos-Cormenzana A. 1992a. Struvite precipitation by soil and fresh water bacteria. Curr Microbiol 24:343–347.
Rivadeneyra MA, Perez-Garcia I, Ramos-Cormenzana A. 1992b. Influence of ammonium ion on bacterial struvite production. Geomicrobiol J 10:125–137.
Rudakov KJ. 1927. Die Reduktion der mineralischen Phosphate auf biologischem Wege. II. Mitteilung. Z Bakt Parasitenk Infektionskr Hyg Abt II 79:229–245.
Ruttenberg KC, Berner RA. 1993. Authigenic apatite formation and burial in sediments from non-upwelling, continental margin environments. Geochim Cosmochim Acta 57:991–1007.
Shapiro J 1967. Induced rapid release and uptake of phosphate by microorganisms. Science 155:1269–1271.

Skinner FA. 1968. The anaerobic bacteria in soil. In: Gray TRG, Parkinson D, eds. The Ecology of Soil Bacteria. Toronto, ON, Canada: Univ Toronto Press, pp 573–592.
Skujins JJ. 1967. Enzymes in soil. In: McLaren AD, Peterson GH, eds. Soil Biochemistry. Vol 1. New York: Marcel Dekker, pp 371–414.
Sperber JI. 1958a. The incidence of apatite-solubilizing organisms in the rhizosphere and soil. Aust J Agric Res 9:778–781.
Sperber JI. 1958b. Release of phosphates from soil minerals by hydrogen sulfide. Nature (Lond) 181:934.
Suess E. 1981. Phosphate regeneration from sediments of the Peru continental margin by dissolution of fish debris. Geochim Cosmochim Acta 45:577–588.
Swaby RJ, Sperber J 1958. Phosphate-dissolving microorganisms in the rhizosphere of legumes. In: Hallsworth EG, ed. Nutrition of Legumes. Proc Univ Nottingham Easter School Agric Sci, 5th. London: Academic Press, pp 289–294.
Taga N, Kobori H. 1978. Phosphatase activity in eutrophic Tokyo Bay. Marine Biol (Berl) 49:223–229.
Tsubota G. 1959. Phosphate reduction in the paddy field. I. Soil Plant Food 5:10–15.
van Groenestijn JW, Deinema MH, Zehnder AJB. 1987. ATP production from polyphosphate in *Acinetobacter* strain 210A. Arch Microbiol 148:14–19.
Varma Ak, Rigsby W, Jordan DC. 1983. A new inorganic pyrophosphate utilizing bacterium from a stagnant lake. Can J Microbiol 29:1470–1474.
Veeh HH, Burnett WC, Soutar A. 1973. Contemporary phosphorites on the continental margin of Peru. Science 181:844–845.
Wackett LP, Shames SL, Venditti CP, Walsh CT. 1987. Bacterial carbon-phosphorus lyase: Products, rates, and regulation of phosphonic and phosphinic acid metabolism. J Bacteriol 169:710–717.
Westheimer FH. 1987. Why nature chose phosphates. Science 235:1173–1178.
Williams BG, Patrick WH Jr. 1971. Effect of E_h and pH on the dissolution of strengite. Nature (Phys Sci) 234:16–17.
Woolfolk CA, Whiteley HR. 1962. Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. I. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements. J Bacteriol 84:647–658.
Youssef MI. 1965. Genesis of bedded phosphates. Econ Geol 60:590–600.

12

Geomicrobially Important Interactions with Nitrogen

12.1 NITROGEN IN THE BIOSPHERE

Nitrogen is an element essential to life. It is abundant in the atmosphere, mostly as dinitrogen (N_2), representing roughly four-fifths by volume or three-fourths by weight of the total gas, the rest being mainly oxygen plus lesser amounts of CO_2 and other common gases. Small amounts of oxides of nitrogen are also present. In soil, sediment, and ocean water, nitrogen exists in both inorganic and organic forms. Geomicrobiologically important inorganic forms include ammonia and ammonium ion, nitrate, and gaseous oxides of nitrogen. Table 12.1 lists the estimated abundance of some of these forms as well as that of organic nitrogen in the environment (see also Stevenson, 1972). Geomicrobially important organic nitrogen compounds include humic and fulvic acids, proteins, peptides, amino acids, purines, pyrimidines, pyridines, other amines, and amides.

Chemically, nitrogen occurs in the oxidation states -3 (e.g., NH_3), -2 (e.g., N_2H_4), -1 (e.g., NH_2OH), 0 (N_2), $+1$ (N_2O), $+2$ (e.g., NO), $+3$ (e.g., HNO_2), $+4$ (e.g., N_2O_4), and $+5$ (e.g., HNO_3). Of these, the -3, -1, 0, $+1$, $+2$, $+3$, and $+5$ oxidation states have biological significance because they can be enzymatically altered. Although nitrogen compounds with nitrogen in the oxidation state of $+4$ are not metabolized by microbes, nitrite formed from the

TABLE 12.1 Abundance of Nitrogen in the Biosphere

Biosphere compartment	Form of nitrogen	Estimated quantity of nitrogen (kg)
Atmosphere	N_2	3.9×10^{18}
Oceans	Organic N	9×10^{14}
	NH_4^+, NO_2^-, NO_3^-	10^{14}
Land	Organic N	8×10^{14}
	NH_4^+, NO_2^-, NO_3^-	1.4×10^{14}
Sediments	Total N	4×10^{17}
Rocks	Total N	1.9×10^{20}
Living biomass	Total N	1.3×10^{13}

Source: Fenchel and Blackburn (1979) and Brock and Madigan (1988).

disproportionation of NO_2 after it is absorbed by soil can be oxidized to nitrate by as yet unidentified organisms (Ghiorse and Alexander, 1978).

Generally, inorganic nitrogen compounds exist in nature either as gases in the atmosphere and dissolved water or as compounds in aqueous solution. Exceptions are small deposits of nitrates of sodium, potassium, calcium, magnesium, and ammonium known as guano or cave, playa, or caliche nitrates (Lewis, 1965). In some cases these nitrate deposits were apparently formed by bacteriological transformation of organic nitrogen formed by nitrogen-fixing bacteria, including cyanobacteria. In other cases they arose from bacterial transformation of organic nitrogen in animal droppings such as those of birds and bats. The organic nitrogen was released as ammonia and then oxidized to nitrate by a consortium of bacteria (Ericksen, 1983) (see Sec. 12.2 for discussion of the reactions). In Chile, deposits of this type, which were probably formed mainly from the microbiota in playa lakes (Ericksen, 1983), are commercially exploited.

Organic nitrogen in nature may exist dissolved in an aqueous phase or in an insoluble state, in the latter case usually in polymers (e.g., certain proteins such as keratin). Insofar as is known, organic nitrogen is usually metabolizable by microbes.

Nitric acid formed through bacterial nitrification can be an important agent in the weathering of rocks and minerals (see Chaps. 4, 6, and 8–10).

12.2 MICROBIAL INTERACTIONS WITH NITROGEN

Ammonification

Most plants derive the nitrogen that they assimilate from soil. This nitrogen is in most instances in the form of nitrate. The nitrate anion is much less readily bound

by mineral soil particles, especially clays, which have a net negative charge, than the ammonium cation. The nitrate supply of the soil depends on recycling of spent organic nitrogen (plant, animal and microbial excretions and remains). The first step in the recycling process is **ammonification**, in which the organic nitrogen is transformed into ammonia. An example of ammonification is the deamination of amino acids:

$$\begin{array}{l} \mathrm{RCHCOOH + NAD^+ \rightarrow RCCOOH + NADH + H^+} \\ \quad\;\; | \qquad\qquad\qquad\quad\;\; \| \\ \quad\;\; \mathrm{NH_2} \qquad\qquad\qquad\quad\;\; \mathrm{NH} \end{array}$$

$$\begin{array}{l} \mathrm{RCCOOH + H_2O \rightarrow RCCOOH + NH_3} \\ \quad\;\; \| \qquad\qquad\qquad\quad\;\; \| \\ \quad\;\; \mathrm{NH} \qquad\qquad\qquad\quad\;\; \mathrm{O} \end{array} \qquad (12.1)$$

The NH_3 reacts with water to form ammonium hydroxide, which dissociates:

$$\mathrm{NH_3 + H_2O \rightarrow NH_4OH \rightarrow NH_4^+ + OH^-} \qquad (12.2)$$

In the laboratory it is commonly observed that when heterotrophic bacteria grow in a proteinaceous medium in which the organic nitrogen serves as the source of energy, carbon, and nitrogen, the pH rises with time due to the liberation of ammonia and its hydrolysis to ammonium ion. Indeed, ammonification is always an essential first step when an amino compound such as an amino acid serves as an energy source.

Ammonia is also formed as a result of urea hydrolysis catalyzed by the enzyme urease:

$$\mathrm{NH_2CONH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}} \qquad (12.3)$$

Urea is a nitrogen waste product excreted in the urine of many mammals. Although urease is produced by a variety of prokaryotic and eukaryotic microbes, a few prokaryotic soil microbes, e.g., *Bacillus pasteurii* and *B. freudenreichii*, seem to be specialists in degrading urea. They prefer to grow at an alkaline pH such as that generated when urea is hydrolyzed (see Alexander, 1977).

Nitrification

Plants can readily assimilate ammonia. But the ammonia produced in ammonification in aqueous systems at neutral pH exists as a positively charged ammonium ion (NH_4^+) due to protonation, which is sorbed by clays and then not readily available to plants. Thus it is important that ammonia be converted into an anionic nitrogen species, which is not readily sorbed by clays and is thus more readily available to plants. *Nitrifying bacteria* play a central role in this

conversion. The majority of nitrifying bacteria are autotrophs and can be divided into two groups; one includes those bacteria that oxidize ammonia to nitrous acid (e.g., *Nitrosomonas*, *Nitrosocystis*), and the second includes those bacteria that oxidize nitrite to nitrate (e.g., *Nitrobacter*, *Nitrococcus*). All the members of these two groups of nitrifying bacteria are obligate autotrophs except for *Nitrobacter winogradskyi* strains, which appear to be facultative. They are all aerobes. Representatives are found in soil, freshwater, and seawater (for further characterization see Paul and Clark, 1996; Balows, 1992).

Although ammonification is the major source of ammonia in soil and sediments, a special anaerobic respiratory process may also be a significant source of ammonia in some environments. In this process, nitrate is reduced to ammonia via nitrite (Jørgensen and Sørensen, 1985, 1988; Binnerup et al., 1992). The overall process can be summarized in the equation

$$NO_3^- + 8(H) + H^+ \rightarrow NH_3 + 3H_2O \tag{12.4}$$

This process is known as **nitrate ammonification** and can be carried on by a number of different facultative and strictly anaerobic bacteria (see, e.g., review by Ehrlich, 1993, pp. 232–233; Dannenberg et al., 1992; Sørensen, 1987). A variety of organic compounds, H_2, and inorganic sulfur compounds can serve as electron donors (H) in this reaction (Dannenberg et al., 1992).

Ammonia Oxidation

Oxidation of ammonia by ammonia-oxidizing bacteria involves hydroxylamine (NH_2OH) as an intermediate (see review by Wood, 1988). The formation of hydroxylamine is catalyzed by an oxygenase:

$$NH_3 + 0.5O_2 \rightarrow NH_2OH \qquad (\Delta G^\circ = +0.77 \text{ kcal}; \ +3.21 \text{ kJ}) \tag{12.5}$$

This reaction does not yield biochemically useful energy. Indeed, it is slightly endothermic and proceeds in the direction of hydroxylamine because it is coupled to the oxidation of hydroxylamine to nitrous acid, which is strongly exothermic. The overall reaction of oxidation of hydroxylamine to HNO_2 can be summarized as

$$NH_2OH + O_2 \rightarrow HNO_2 + H_2O \qquad (\Delta G^\circ = -62.42 \text{ kcal}; \ -260.9 \text{ kJ}) \tag{12.6}$$

It is reaction (12.6) from which chemoautotrophic ammonia oxidizers obtain their energy, by using chemiosmotic coupling, i.e., oxidative phosphorylation. The conversion of hydroxylamine to nitrous acid involves some intermediate steps (Hooper, 1984).

The enzyme that catalyzes ammonia oxidation [reaction (12.5)] is a nonspecific oxygenase. It can also catalyze the oxygenation of methane to methanol (Jones and Morita, 1983):

$$CH_4 + 0.5O_2 \rightarrow CH_3OH \qquad (\Delta G^\circ = -29.74 \text{ kcal}; \ -124.3 \text{ kJ}) \qquad (12.7)$$

Under standard conditions, this reaction is thermodynamically more favorable than reaction (12.5). This does not mean, however, that ammonia oxidizers can grow on methane. They lack the ability to oxidize methanol.

Ammonia oxidizers can also form some NO and N_2O in side reactions of ammonia oxidation under oxygen limitation in which nitrite replaces oxygen as terminal electron acceptor (see discussions by Knowles, 1985; Bock et al., 1991; Davidson, 1993). This is an important observation because it means that biogenically formed N_2O and NO are not solely the result of denitrification (see next section).

Nitrite Oxidation

The nitrite oxidizers convert nitrite to nitrate:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (\Delta G^\circ = -18.18 \text{ kcal}; \ -76.0 \text{ kJ}) \qquad (12.8)$$

They obtain useful energy from this process by coupling it chemiosmotically to ATP generation (Aleem and Sewell, 1984; Wood, 1988).

Heterotrophic Nitrification

Ammonia can also be converted to nitrate by certain heterotrophic microorganisms, but the process is probably of minor importance in nature in most instances. Rates of heterotrophic nitrification measured under laboratory conditions so far are significantly slower than those of autotrophic nitrification. The organisms capable of heterotrophic nitrification include both bacteria such as *Arthrobacter* sp. and fungi such as *Aspergillus flavus*. They gain no energy from the conversion. The pathway from ammonia to nitrate may involve intermediates such as hydroxylamine, nitrite, and 1-nitrosoethanol in the case of bacteria and 3-nitropropionic acid in the case of fungi (see Alexander, 1977; Paul and Clark, 1996).

Anaerobic Ammonia Oxidation

Recent evidence indicates that one or more bacteria, as yet unidentified, can oxidize ammonium (NH_4^+) anaerobically to dinitrogen (N_2). A disproportionation reaction with nitrates is probably involved (Van de Graaf et al., 1995):

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$$
$$(\Delta G^{\circ\prime} = -354.6 \text{ kcal}; \ -1483.5 \text{ kJ}) \qquad (12.9)$$

This reaction can also be viewed as a form of denitrification (see below). As the discoverers of this reaction indicate, it yields enough energy for some of it to be conserved by active organisms.

Denitrification

Nitrate, nitrite, and nitrous and nitric oxides can serve as electron acceptors in microbial respiration, usually under anaerobic conditions. The transformation of nitrate to nitrite is called *dissimilatory nitrate reduction*, and the reduction of nitrate to nitric oxide (NO), nitrous oxide (N_2O), and/or dinitrogen is called **denitrification**. *Assimilatory nitrate reduction* is the first step in a process in which nitrate is reduced to ammonia for the purpose of assimilation. Only as much nitrate is consumed in this process as is needed for assimilation. It is not a form of respiration and is performed by many organisms that cannot use nitrate for respiration. Some nitrate-respiring bacteria are capable of only nitrate reduction, lacking the enzymes for reduction of nitrite to dinitrogen, whereas others are capable of reducing nitrite to ammonia instead of dinitrogen in a process that has been called nitrate ammonification by Sørensen (1987) (see also discussion in previous section). All nitrate respiratory processes have been found to operate to varying degrees in terrestrial, freshwater, and marine environments and represent an important part of the nitrogen cycle favored by anaerobic conditions.

Nitrate reduction is described by the half-reaction

$$NO_3^- + 2H^+ + 2e \rightarrow NO_2^- + H_2O \tag{12.10}$$

The electron donor may be any one of a variety of organic metabolites or reduced sulfur such as H_2S or S^0. The enzyme catalyzing reaction (12.10) is called *nitrate reductase* and is an iron molybdoprotein. It is not only capable of catalyzing nitrate reduction but may also catalyze reduction of ferric to ferrous iron (see Chap. 15) and reduction of chlorate to chlorite. Nitrate can competitively affect ferric iron reduction by nitrate reductase (Ottow, 1969).

Nitrite may be reduced to dinitrogen by the following series of half-reactions, with organic metabolites or reduced sulfur acting as electron donor:

$$NO_2^- + 2H^+ + e \rightarrow NO + H_2O \tag{12.11}$$

$$2NO + 2H^+ + 2e \rightarrow N_2O + H_2O \tag{12.12}$$

$$N_2O + 2H^+ + 2e \rightarrow N_2 + H_2O \tag{12.13}$$

The reduction of nitrite to ammonia may be summarized by the equation

$$NO_2^- + 7H^+ + 6e \rightarrow NH_3 + 2H_2O \tag{12.14}$$

The electron donor may be one of a variety of organic metabolites.

Although it was previously believed that these reactions can occur only at low oxygen tension or in the absence of oxygen, evidence now indicates that in some cases organisms such as *Thiosphaera pantotropha* can perform the reactions at near normal oxygen tension (Robertson and Kuenen, 1984a, 1984b; but see also disagreement by Thomsen et al., 1993). This organism can actually use oxygen and nitrate simultaneously as terminal electron acceptors. The explanation is that in *Tsa. pantotropha* the enzymes of denitrification are produced aerobically as well as anaerobically, whereas in oxygen-sensitive denitrifiers they are produced only at low oxygen tension or anaerobically. Nitrate reductase in *Tsa. pantotropha* is constitutive, whereas in many anaerobic denitrifiers it is inducible. Moreover, nitrate reductase in *Tsa. pantotropha* is not inactivated by oxygen, as in some anaerobic denitrifiers. Finally, oxygen does not repress formation of the denitrifying enzymes in *Tsa. pantotropha*, as it does in some anaerobic denitrifiers. Aerobic denitrification in this organism appears to be linked to heterotrophic nitrification (Robertson et al., 1988; Robertson and Kuenen, 1990). The organism seems to use denitrification as a means of disposing of excess reducing power because its cytochrome system is insufficient for this purpose. Oxygen tolerance in denitrification has also been observed with some other bacteria (Hochstein et al., 1984; Davies et al., 1989; Bonin et al., 1989).

For a more complete discussion of denitrification, the reader is referred to a monograph by Payne (1981) and a review article by the same author (Payne, 1983).

Nitrogen Fixation

If nature had not provided for microbial **nitrogen fixation** to reverse the effect of microbial depletion of fixed nitrogen from soil or water as volatile nitrogen oxides or of dinitrogen by denitrification, life on Earth would not have long continued after the process of denitrification first evolved. Nitrogen fixation is dependent on a special enzyme, *nitrogenase*, which is found only in prokaryotic organisms, including aerobic and anaerobic photosynthetic and nonphotosynthetic Bacteria and Archaea. Nitrogenase is an oxygen-sensitive enzyme, usually a combination of an iron protein and a molybdoprotein (Eady and Postgate, 1974; Orme-Johnson, 1992), but in some cases (e.g., *Azotobacter chroococcum*) may also be a combination of an iron protein and a vanadoprotein (Robson et al., 1986; Eady et al., 1987), and in another case (*Azotobacter vinelandii*) may be a combination of two iron proteins (Chiswell et al., 1988). Nitrogenase catalyzes the reaction

$$N_2 + 6H^+ + 6e \rightarrow 2NH_3 \tag{12.15}$$

The enzyme is not specific for dinitrogen. It can also catalyze the reduction of acetylene, CH≡CH, as well as of hydrogen cyanide (HCN), cyanogen (NCCN),

hydrogen azide (HN_3), hydrogen thiocyanate (HCNS), protons (H^+), carbon monoxide (CO), and some other compounds (Smith, 1983).

The reducing power needed for dinitrogen reduction is provided by reduced ferredoxin. Reduced ferredoxin can be formed in a reaction in which pyruvate is oxidatively decarboxylated (e.g., Lehninger, 1975):

$$CH_3COCOOH + NAD^+ + CoASH \rightarrow CH_3CO \sim SCoA + CO_2 + NADH + H^+ \quad (12.16)$$

$$NADH + H^+ + (\text{ferredoxin})_{ox} \rightarrow NAD^+ + (\text{ferredoxin})_{red} + 2H^+ \quad (12.17)$$

In phototrophs, the reduced ferredoxin is produced as part of the photophosphorylation mechanism (see Chap. 6).

Nitrogen fixation is a very energy-intensive reaction, consuming as many as 16 moles of ATP per mole of dinitrogen in the reduction of dinitrogen to ammonia (Newton and Burgess, 1983).

Nitrogen fixation may proceed symbiotically or nonsymbiotically. *Symbiotic nitrogen fixation* requires that the nitrogen-fixing bacterium associate with a specific host plant (e.g., a legume), one of several nonleguminous angiosperms, the water fern *Azolla*, fungi (certain lichens), or, in rare cases, with an animal host in order to carry out nitrogen fixation. Even then, dinitrogen will be fixed only if the fixed-nitrogen level in the surrounding environment of the host plant is low or the diet of the animal host is nitrogen-deficient. In some plants (e.g., legumes or alder), the nitrogen fixer may be localized in cells of the cortical root tissue that are transformed into nodules. Invasion of the plant tissue may have occurred via root hairs. In some other plants the nitrogen fixers may be localized in special leaf structures (e.g., in *Azolla*). In animals, the nitrogen fixer may be found to be a member of the flora of the digestive tract (Knowles, 1978). The plant host in symbiotic nitrogen fixation provides the energy source required by the nitrogen fixer for generating ATP. The energy source may take the form of compounds such as succinate, malate, and fumarate (Paul and Clark, 1996). The plant host also provides an environment in which access to oxygen is controlled so that nitrogenase in a nitrogen fixer is not inactivated. In root nodules of legumes, leghemoglobin is involved in this control of oxygen. The nitrogen fixer shares the ammonia that it forms from dinitrogen with its plant or animal host.

Symbiotic nitrogen-fixing bacteria include members of the genera *Rhizobium*, *Bradyrhizobium*, *Frankia*, and *Anabaena*. Some strains of *B. japonicum* have been found to be able to grow autotrophically on hydrogen as energy source because they possess uptake hydrogenase. They can couple hydrogen oxidation to ATP synthesis, which they can use in CO_2 assimilation via the ribulose bisphosphate carboxylase/oxidase system. In nitrogen fixation, the ability to couple hydrogen oxidation to ATP synthesis may represent an energy conservation system because nitrogenase generates a significant amount of hydrogen during

nitrogen fixation, the energy content of which would otherwise be lost to the system.

About 25 years ago, a special symbiotic nitrogen-fixing relationship was discovered in Brazil between certain cereal grasses, such as maize, and nitrogen-fixing spirilla, such as *Azospirillum lipoferum* (Von Bülow and Döbereiner, 1975; Day et al., 1975; Smith et al., 1976). In these symbioses, the bacterium does not invade the plant roots or any other part of the plant but lives in close association with them in the **rhizosphere**. Apparently the plants excrete compounds that the bacterium can use as energy sources and that enable it to fix nitrogen that it can share with the plant if the soil is otherwise deficient in fixed nitrogen.

In *nonsymbiotic nitrogen fixation*, the active organisms are free-living in soil or water and fix nitrogen if fixed nitrogen is limiting. Their nitrogenase is not distinctly different from that of symbiotic nitrogen fixers. Unlike the symbiotic nitrogen fixers, nonsymbiotic nitrogen fixers appear to be able to maintain an intracellular environment in which nitrogenase is not inactivated by oxidizing conditions. The capacity for nonsymbiotic nitrogen fixation is widespread among prokaryotes. The best known examples and the most efficient include the aerobes *Azotobacter* and *Beijerinckia* and the anaerobe *Clostridium pasteurianum*, but many other aerobic and anaerobic genera include species with nitrogen-fixing capacity, even some photo- and chemolithotrophs. Most of the nitrogen fixers are active only at environmental pH values between 5 and 9, but some strains of the acidophile *Thiobacillus ferrooxidans* have been shown to fix nitrogen at a pH as low as 2.5.

For a more detailed discussion of nitrogen fixation, the reader is referred to Alexander (1984), Broughton (1983), Newton and Orme-Johnson (1980), and Quispel (1974).

12.3 MICROBIAL ROLE IN THE NITROGEN CYCLE

Owing to their special capacities for transforming inorganic nitrogen compounds, which plants and animals lack, microbes, especially prokaryotes and certain fungi, play a central role in the nitrogen cycle (Fig. 12.1). Many reactions of the cycle are entirely dependent on them; nitrogen fixation is restricted to prokaryotes. The direction of transformations in the cycle is determined by environmental conditions, especially the availability of oxygen, but also by the supply of particular nitrogen compounds. Anaerobic conditions may encourage denitrification and thus cause nitrogen limitation unless the process is counteracted by anaerobic nitrogen fixation. Limitations in the supply of organic or ammonia nitrogen affect the availability of nitrate. Availability of fixed nitrogen is frequently a growth-limiting factor in the marine environment but infrequently

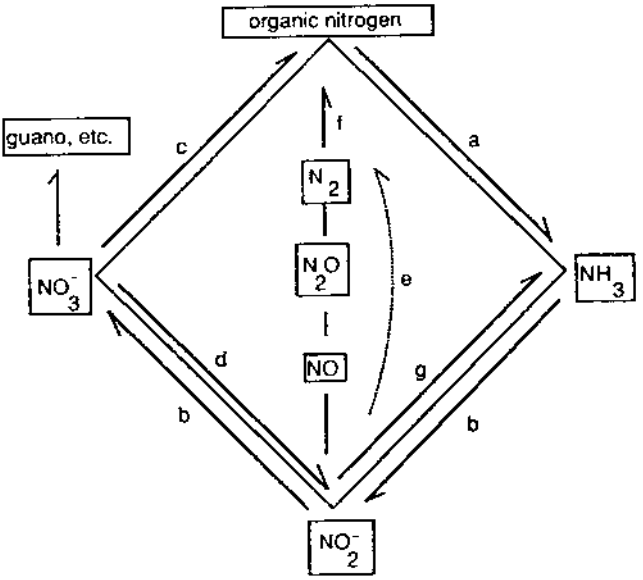


FIG. 12.1 The nitrogen cycle. (a) Ammonification (aerobic and anaerobic), (b) autotrophic nitrification (strictly aerobic), (c) nitrate assimilation (aerobic or anaerobic), (d) nitrate reduction [usually anaerobic, but see (e)], (d,e) denitrification (usually anaerobic but sometimes aerobic; see text), (f) nitrogen fixation (aerobic and anaerobic), (d,g) nitrate ammonification.

in unpolluted freshwater, where phosphate is more likely to limit productivity. Fixed nitrogen can be a limiting factor in soil, especially in agriculturally exploited soils.

12.4 SUMMARY

Nitrogen is essential to all forms of life. It is assimilated by cells in the form of ammonia and/or nitrate. It is released from organic combination in the form of ammonia. The latter process is called ammonification. It occurs both aerobically and anaerobically. Ammonia is an energy-rich compound and can be oxidized to nitrate by way of nitrite by some aerobic, autotrophic bacteria (nitrifiers). It can also be converted to nitrate by some heterotrophic bacteria and some fungi in a non-energy-yielding process, but this is much less common. The conversion of ammonia to nitrate is important in soil and sediments because negatively charged clay particles can adsorb ammonia, making it unavailable to plants. Under reducing conditions, nitrate can be transformed by anaerobic respiration to nitrite,

nitric and nitrous oxides, dinitrogen, or ammonia by appropriate bacteria. In soil the reduction of nitrate to dinitrogen can have the effect of lowering its fertility. Depletion of soil nitrogen through dinitrogen evolution can, however, be reversed by symbiotic and nonsymbiotic nitrogen-fixing bacteria, which are able to reduce dinitrogen to ammonia. These various biological interactions are part of a cycle that is essential to the sustenance of life on Earth.

REFERENCES

Aleem MIH, Sewell DL. 1984. Oxidoreductase systems in *Nitrobacter agilis*. In: Strohl WR, Tuovinen OH, eds. Microbial Chemoautotrophy. Columbus, OH: Ohio State Univ Press, pp 185–210.

Alexander M. 1977. Introduction to Soil Microbiology. 2nd ed. New York: Wiley.

Alexander M, ed. 1984. Biological Nitrogen Fixation: Ecology, Technology, and Physiology. New York: Plenum.

Balows A, ed. 1992. The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. 2nd ed. New York: Springer-Verlag.

Binnerup SJ, Jensen K, Revsbech NP, Jensen MH, Sørensen J. 1992. Denitrification, dissimilatory reduction of nitrate to ammonium, and nitrification in a bioturbated estuarine sediment as measured with ^{15}N and microsensor techniques. Appl Environ Microbiol 58:303–313.

Bock E, Koops H-P, Harms H, Ahlers B. 1991. The biochemistry of nitrifying organisms. In: Shively JM, Barton LL, eds. Variations in Autotrophic Life. London: Academic Press, pp 171–200.

Bonin P, Gilewicz M, Bertrand JC. 1989. Effects of oxygen on each step of denitrification on *Pseudomonas nautica*. Can J Microbiol 35:1061–1064.

Brock TD, Madigan MT. 1988. Biology of Microorganisms. 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Broughton WJ, ed. 1983. Nitrogen Fixation, Vol I, Ecology; Vol 2, Rhizobium; Vol 3, Legumes. Oxford, UK: Clarendon Press.

Chiswell JR, Premarkumar R, Bishop PE. 1988. Purification of a second alternative nitrogenase from a *nif* HDK deletion strain of *Azotobacter vinelandii*. J Bacteriol 170:27–33.

Dannenberg S, Kroder M, Dilling W, Cypionka H. 1992. Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch Microbiol 158:93–99.

Davidson EA. 1993. Soil water content and the ratio of nitrous to nitric oxide emitted from soil. In: Oremland RS, ed. Biogeochemistry of Global Change. Radiatively Active Trace Gases. New York: Chapman and Hall, pp 369–386.

Davies KJP, Lloyd D, Boddy L. 1989. The effect of oxygen on denitrification in *Paracoccus denitrificans* and *Pseudomonas aeruginosa*. J Gen Microbiol 135:2445–2451.

Day JM, Neves MCP, Döbereiner J. 1975. Nitrogenase activity on the roots of tropical forage grasses. Soil Biol Biochem 7:107–112.

Eady RR, Postgate JR. 1974. Nitrogenase. Nature (Lond) 249:805–810.

Eady RR, Robson RL, Richardson TH, Miller RW, Hawkins M. 1987. The vanadium nitrogenase of *Azotobacter chroococcum*. Purification and properties of the vanadium-iron protein. Biochem J 244:197–207.

Ehrlich HL. 1993. Bacterial mineralization of organic matter under anaerobic conditions. In: Bollag J-M, Stotzky G, eds. Soil Biochemistry, Vol 8. New York: Marcel Dekker, pp 219–238.

Ericksen GE. 1983. The Chilean nitrate deposits. Am Sci 71:366–374.

Fenchel T, Blackburn TH. 1979. Bacteria and Mineral Cycling. London: Academic Press.

Ghiorse WC, Alexander M. 1978. Nitrifying populations and the destruction of nitrogen dioxide in soil. Microb Ecol 4:233–240.

Hochstein LI, Betlach M, Kritikos K. 1984. The effect of oxygen on denitrification during steady-state growth of *Paracoccus halodenitrificans*. Arch Microbiol 137:74–78.

Hooper AB. 1984. Ammonia oxidation and energy transduction in the nitrifying bacteria. In: Strohl WR, Tuovinen OH, eds. Microbial Chemoautotrophy. Columbus, OH: Ohio State Univ Press, pp 133–167.

Jones RD, Morita RY. 1983. Methane oxidation by *Nitrosococcus oceanus* and *Nitrosomonas europaea*. Appl Environ Microbiol 45:401–410.

Jørgensen BB, Sørensen J. 1985. Seasonal cycles of O_2, NO_3^- and SO_4^{2-} reduction in estuarine sediments: The significance of a NO_3^- reduction maximum in the spring. Mar Ecol Progr Ser 24:65–74.

Jørgensen BB, Sørensen J. 1988. Two annual maxima of nitrate reduction and denitrification in estuarine sediment (Norsminde Fjord, Denmark). Mar Ecol Progr Ser 48:147–154.

Knowles R. 1978. Free-living bacteria. In: Dörbereiner R, Burris H, Hollaender A, Franco AA, Neyra CA, Scott DB, eds. Limitations and Potentials for Biological Nitrogen Fixation in the Tropics. New York: Plenum Press, pp 25–40.

Knowles R. 1985. Microbial transformations as sources and sinks of nitrogen oxides: In: Caldwell DE, Brierley JA, Brierley CL, eds. Planetary Ecology. New York: Van Nostrand Reinhold, pp 411–426.

Lehninger AL. 1975. Biochemistry. 2nd ed. New York: Worth.

Lewis RW. 1965. Nitrogen. In: Mineral Facts and Problems, Bull 630. Washington, DC: US Bur Mines, pp 621–629.

Newton WE, Burgess BK. 1983. Nitrogen fixation: Its scope and importance. In: Mueller A, Newton WE, eds. Nitrogen Fixation. The Chemical-Biochemical-Genetic Interface. New York: Plenum, pp 1–19.

Newton WE, Orme-Johnson WH, eds. 1980. Nitrogen Fixation. Baltimore: Univ Park Press.

Orme-Johnson WH. 1992. Nitrogenase structure: Where to now? Science 257:1639–1640.

Ottow JCG. 1969. Der Einfluss von Nitrat, Chlorat, Sulfat, Eisenoxyd-form und Wachstumsbedingungen auf das Ausmass der Baketeriellen Eisenreduktion. Z Pflanzenernähr Düngung Bodenk 124:238–253.

Paul EA, Clark PF. 1996. Soil Microbiology and Biochemistry. San Diego: Academic Press.

Payne WJ. 1981. Denitrification. New York: Wiley.
Payne WJ. 1983. Bacterial denitrification: Asset or defect. BioScience 33:319–325.
Quispel A, ed. 1974. The Biology of Nitrogen Fixation. Amsterdam: North-Holland.
Robertson LA, Kuenen JG. 1984a. Aerobic denitrification: A controversy revived. Arch Microbiol 139:351–354.
Robertson LA, Kuenen JG. 1984b. Aerobic denitrification: Old wine in new bottles? Antonie v Leeuwenhoek 50:525–544.
Robertson LA, Kuenen JG. 1990. Combined heterotrophic nitrification and aerobic denitrification in *Thiosphaera pantotropha* and other bacteria. Antonie v Leeuwenhoek 57:139–152.
Robertson LA, van Niel EWJ, Torremans RAM, Kuenen JG. 1988. Simultaneous nitrification and denitrification in aerobic chemostat cultures of *Thiosphaera pantotropha*. Appl Environ Microbiol 54:2812–2818.
Robson RL, Eady RR, Richardson TH, Miller RW, Hawkins M, Postgate JR. 1986. The alternative nitrogenase of *Azotobacter chroococcum* is a vanadium enzyme. Nature (Lond) 322:388–390.
Smith BE. 1983. Reactions and physicochemical properties of the nitrogenase MoFe proteins. In: Mueller A, Newton WE, eds. Nitrogen Fixation. The Chemical-Biochemical-Genetic Interface. New York: Plenum, pp 23–62.
Smith RL, Bouton JH, Schank SC, Quesenberry KH, Tyler ME, Milam JR, Gaskin MH, Littell RC. 1976. Nitrogen fixation in grasses inoculated with *Spirillum lipoferum*. Science 193:1003–1005.
Sørensen J. 1987. Nitrate reduction in marine sediment: Pathway and interactions with iron and sulfur recycling. Geomicrobiol J 5:401–421.
Stevenson FJ. 1972. Nitrogen: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 795–801.
Thomsen JK, Iversen JJL, Cox RP. 1993. Interactions between respiration and denitrification during growth of *Thiosphaera pantotropha* in continuous culture. FEMS Microbiol Lett 110:319–324.
Van de Graaf AA, Mulder A, de Bruijn P, Jetten MSM, Robertson LA, Kuenen JG. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. Appl Environ Microbiol 61:1246–1251.
Von Bülow JFW, Döbereiner J. 1975. Potential for nitrogen fixation in maize genotypes in Brazil. Proc Natl Acad Sci USA 72:2389–2393.
Wood P. 1988. Chemolithotrophy. In: Anthony C, ed. Bacterial Energy Transduction. London: Academic Press, pp 183–230.

13

Geomicrobial Interactions with Arsenic and Antimony

13.1 INTRODUCTION

Although arsenic and antimony are generally toxic to life, some microorganisms exist that can metabolize some forms of these elements. Some can use arsenite or stibnite as partial or sole energy sources while others can use arsenate as the electron acceptor. Still other microbes can metabolize arsenic and antimony compounds to detoxify them. These reactions are important from a geomicrobial standpoint because they indicate that some microbes contribute to arsenic and antimony mobilization or immobilization in the environment and play a role in arsenic and antimony cycles.

13.2 ARSENIC

Distribution

Arsenic is widely distributed in the upper crust of the Earth, mostly at very low concentration. Its average abundance in igneous rocks has been estimated to be of the order of 5 g tonne^{-1} (Carapella, 1972). It rarely occurs in elemental form. More often it is found combined with sulfur, as in orpiment (As_2S_3), realgar (AsS), or arsenopyrite (FeAsS); with selenium, as in As_2Se_3; with tellurium, as in As_2Te; or as sulfosalt, as in enargite (Cu_3AsS_4). It is also found in arsenides of

heavy metals such as iron (loellingite, $FeAs_2$), copper (domeykite, Cu_3As), nickel (nicolite, NiAs), and cobalt (Co_2As). Sometimes the element occurs in the form of arsenite minerals (arsenolite or claudetite, As_2O_3) or in the form of arsenate minerals [erythrite, $Co_3(AsO_4)_2 \cdot 8H_2O$; scorodite, $FeAsO_4 \cdot 2H_2O$; olivenite, $Cu_2(AsO_4)(OH)$]. Arsenopyrite is the most common and widespread mineral form of arsenic, but orpiment and realgar are also fairly common. The ultimate source of arsenic on the Earth's surface is igneous activity. On weathering of arsenic-containing rocks, which may harbor as much as 1.8 ppm of the element, the arsenic is dispersed through the upper lithosphere and the hydrosphere.

Arsenic concentration in soil may range from 0.1 to more than 1000 ppm. The average concentration in seawater has been reported to be $3.7\,\mu g\,L^{-1}$ and in freshwater, $0.5\,\mu g\,L^{-1}$. In air, arsenic may be found in a concentration range of $1.5–53\,ng\,m^{-3}$ (Bowen, 1979). Some living organisms may concentrate arsenic manyfold over its level in the environment. For example, some algae have been found to accumulate arsenic 200–3000 times in excess of its concentration in the growth medium (Lunde, 1973). Humans may artificially raise the arsenic concentrations in soil and water through the introduction of sodium arsenite (Na_3AsO_3) or cacodylic acid [$(CH_3)_2AsO \cdot OH$] as herbicide.

Some Chemical Characteristics

The oxidation states in which arsenic is usually encountered in nature include 0, +3, and +5. Its coordination numbers are in the range of 3, 4, 5, and 6 (Cullen and Reimer, 1989). Except for the oxidation state of As in arsenate and arsenite, the oxidation state in other compounds, whether organic or inorganic, is often unclear and depends on its definition (Cullen and Reimer, 1989, p. 715). In aqueous solution, arsenious acid and its salts exist in the ortho (H_3AsO_3) but not the meta ($HAsO_2$) form (see Cullen and Reimer, 1989, p. 715).

Toxicity

Arsenic compounds are toxic to most living organisms. Arsenite (AsO_3^{3-}) has been shown to inhibit dehydrogenases such as pyruvate, α-ketoglutarate, and dihydrolipoate dehydrogenases (Mahler and Cordes, 1966). Arsenate (AsO_4^{3-}) uncouples oxidative phosphorylation, i.e., it inhibits ATP synthesis by chemiosmosis (Da Costa, 1972).

Both the uptake of arsenate and the inhibitory effect of arsenate on metabolism can be modified by phosphate (Button et al., 1973; Da Costa, 1971, 1972). This is because a common transport mechanism for phosphate and arsenate exists in the membranes of some organisms. However, a separate transport mechanism for phosphate may also exist (Bennett and Malamy, 1970). In the latter case, phosphate uptake does not affect arsenate uptake, nor does

arsenate uptake affect phosphate uptake. In one reported case, that of a fungus, *Cladosporium herbarium*, arsenite toxicity could also be ameliorated by the presence of phosphate. In that instance, prior oxidation of the arsenite to arsenate by the fungus appeared to be the basis for the effect (Da Costa, 1972). In growing cultures of *Candida humicola*, phosphate can inhibit the formation of trimethylarsine from arsenate, arsenite, and monomethylarsonate, but not from dimethylarsinate (Cox and Alexander, 1973). In similar cultures, phosphite can suppress the formation of trimethylarsine from monomethylarsonate but not from arsenate or dimethylarsinate. Hypophosphite can cause temporary inhibition of the conversion of arsenate, monomethylarsonate, and dimethylarsinate (Cox and Alexander, 1973). High antimonate concentrations lower the rate of conversion of arsenate to trimethylarsine by resting cells of *Candida humicola* (Cox and Alexander, 1973).

Bacteria can develop genetically determined resistance to arsenic (Ji and Silver, 1992; Ji et al., 1993). This resistance can be determined by a gene locus on a plasmid, for example, in *Staphylococcus aureus* (Dyke et al., 1970) and *Escherichia coli* (Hedges and Baumberg, 1973). The mechanism of resistance in these bacterial species is a special pumping mechanism that expels the arsenic taken up as arsenate by the cells (Silver and Keach, 1982). It involves reduction of arsenate to arsenite intracellularly followed by efflux of the arsenite promoted by an oxyanion translocating ATPase (Broeer et al., 1993; Ji et al., 1993). Some of the resistant organisms have the capacity to oxidize reduced forms of arsenic to arsenate and others to reduce oxidized forms of arsenic to reduced forms (see below).

Microbial Oxidation of Reduced Forms of Arsenic

Dissolved Arsenic Species

Bacterial oxidation of arsenite to arsenate was first reported by Green (1918). He discovered an organism with this ability in arsenical cattle-dipping solution used for protection against insect bites. He named his isolated organism *Bacillus arsenoxydans*. Quastel and Scholefield (1953) observed arsenite oxidation in perfusion experiments in which they passed 2.5×10^{-3} M sodium arsenite solution through columns charged with Cardiff soil. They did not isolate the organism or organisms responsible for the oxidation but showed that a 0.1% solution of NaN_3 inhibited the oxidation. The onset of arsenite oxidation in their experiments occurred after a lag. The length of this lag was increased when sulfanilamide was added with the arsenite. A control of pH was found important for sustained bacterial activity. They observed an almost stoichiometric O_2 consumption during arsenite oxidation.

Further investigation of arsenical cattle-dipping solutions led to the isolation of 15 arsenite-oxidizing strains of bacteria (Turner, 1949, 1954). These organisms were assigned to the genera *Pseudomonas*, *Xanthomonas*, and *Achromobacter*. *Achromobacter arsenoxydans-tres* was later considered synonymous with *Alcaligenes faecalis* (Hendrie et al., 1974). This organism is described in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) as frequently having the capacity for arsenite oxidation.

Of Turner's 15 isolates, *Pseudomonas arsenoxydans-quinque* was studied in detail with respect to arsenite oxidation. Resting cells of this culture oxidized arsenite at an optimum pH of 6.4 and at an optimum temperature of 40°C (Turner and Legge, 1954). Cyanide, azide, fluoride, and pyrophosphate inhibited the activity. Under anaerobic conditions, 2,6-dichlorophenol indophenol, *m*-carboxyphenolindo-2,6-dibromophenol, and ferricyanide could act as electron acceptors. Pretreating the cells with toluene and acetone or desiccating them rendered them incapable of oxidizing arsenite in air. The arsenite-oxidizing enzyme was "adaptable." Examination of arsenite oxidation by cell-free extracts of *P. arsenoxidans-quinque* suggested the presence of soluble "dehydrogenase" activity, which under anaerobic conditions conveyed electrons from arsenite to 2,6-dichlorophenol indophenol (Legge and Turner, 1954). The activity was partly inhibited by 10^{-3} M *p*-chloromercuribenzoate. The entire arsenite-oxidizing system was believed to consist of "dehydrogenase" and an oxidase (Legge, 1954).

A soil strain of *Alcaligenes faecalis* was isolated by Osborne (1973) whose arsenite-oxidizing ability was found to be inducible by arsenite and arsenate (Osborne and Ehrlich, 1976). Very recent phylogenetic study of this strain by 16S rDNA analysis showed that it should be reassigned to the genus *Achromobacter* (Santini, personal communication, 2001). It was shown to oxidize arsenite stoichiometrically to arsenate (Table 13.1)*:

$$AsO_2^- + H_2O + 0.5O_2 \rightarrow AsO_4^{3-} + 2H^+ \qquad (13.1)$$

Inhibitor and spectrophotometric studies suggested that the oxidation involved an oxidoreductase with a bound flavin that passed electrons from arsenite to oxygen by way of a c-type cytochrome and cytochrome oxidase (Osborne, 1973; Osborne and Ehrlich, 1976).

Anderson et al. (1992) isolated an inducible arsenite-oxidizing enzyme that was located on the outer surface of the plasma membrane of *A. faecalis* strain

* The formula for *meta*-arsenite is used in this equation even though *ortho*-arsenite is the form of As(III) in aqueous solution. The equation as shown conveys that the reaction mixture turns acid, if unbuffered, as the oxidation progresses. The increase in acidity results from the fact that arsenious acid is a much weaker acid ($K = 10^{-9.2}$) than arsenic acid ($K_1 = 10^{-2.25}$; $K_2 = 10^{-6.77}$; $K_3 = 10^{-11.4}$) (Weast and Astle, 1982).

TABLE 13.1 Stoichiometry of Oxygen Uptake by *Alcaligenes faecalis* on Arsenite Based on the Reaction of $AsO_2^- + H_2O + 1/2O_2 \rightarrow AsO_4^{3-} + 2H^+$

	Oxygen uptake		
$NaAsO_2$ added (μmol)	Theoretical	Experimental	Percent of theoretical
19.25	9.63	8.79	91.3
38.50	19.25	18.48	96.0
57.75	28.88	27.05	93.7
77.00	38.50	37.05	96.2

Source: Osborne (1973), with permission.

NCIB 8687. The enzyme location suggests that in intact cells of this organism, arsenite oxidation occurs in its periplasmic space. Biochemical characterization showed the enzyme to be a molybdenum-containing hydroxylase consisting of a monomeric 85 kDa peptide with a pterin cofactor and one atom of molybdenum, five or six atoms of iron, and inorganic sulfide. Both azurin and cytochrome c from *A. faecalis* served as electron acceptors in arsenite oxidation catalyzed by this enzyme.

A strain of *A. faecalis* similar to the one isolated by Osborne (1973) (see also Osborne and Ehrlich, 1976) was independently isolated and characterized by Phillips and Taylor (1976). Neither their strain nor that of Osborne oxidized arsenite strongly until late exponential phase or stationary phase of growth was reached in batch culture (Phillips and Taylor, 1976; Ehrlich, 1978). Other heterotrophic arsenite-oxidizing bacteria that have been identified more recently include *Pseudomonas putida* strain 18 and *Alcaligenes eutrophus* strain 280, both of which were isolated from gold-arsenic deposits (Abdrashitova et al., 1981) and a strain that appears to belong to the genus *Zoogloea* (Weeger et al., 1999).

The observation reported by Osborne and Ehrlich (1976) that their strain passes electrons from arsenite to oxygen via an electron transport system that involves c-type cytochrome and cytochrome oxidase suggested that their organism is able to conserve energy from this oxidation. Indeed, indirect evidence indicated that the organism may be able to derive maintenance energy from arsenite oxidation (Ehrlich, 1978). Much stronger evidence that arsenite can be used as an energy source by some arsenite-oxidizing bacteria was presented by Ilyaletdinov and Abdrashitova (1981), who isolated a culture, which they named *Pseudomonas arsenitoxidans*, from a gold-arsenic ore deposit. The culture was an obligate autotroph that grew on arsenite as sole energy source. It also oxidized arsenic in arsenopyrite. Recently Santini et al. (2000) reported the isolation of two new strains of autotrophic arsenite oxidizers, N-25 and N-26, from a gold mine in the Northern Territory of Australia. Both strains belong to the *Agrobacterium-*

Rhizobium branch of the α-proteobacteria. Growth of strain N-26 was accelerated by a trace of yeast extract added to an arsenite–mineral salts growth medium. Strain N-26 was also able to grow heterotrophically on a wide range of organic carbon/energy sources including glucose, fructose, succinate, fumarate, pyruvate, and several others. This strain thus appears to be facultatively autotrophic, being also able to grow mixotrophically and heterotrophically. The arsenite oxidase of strain N-26 appears to be periplasmic.

Arsenite [As(III)] may under some conditions also be subject to abiotic oxidation by Mn(IV), but apparently to a much lesser extent or not at all by Fe(III) (Oscarson et al., 1981).

Arsenic-Containing Minerals

Arsenic in minerals that also contain iron, copper, and/or sulfur may be mobilized by bacteria. This mobilization may or may not involve bacterial oxidation of the arsenic. The simplest of these compounds, orpiment (As_2S_3), was found to be oxidized by *Thiobacillus* (now renamed *Acidithiobacillus*) *ferrooxidans* TM in a mineral salts solution (9K medium without iron; for formulation see Silverman and Lundgren, 1959) to which the mineral had been added in pulverized form (Ehrlich, 1963) (Fig. 13.1). Arsenite and arsenate accumulated in the medium over time. Because *T. ferrooxidans* does not oxidize arsenite to arsenate, the arsenate that appeared in the inoculated flasks probably resulted from autoxidation of arsenite produced by the organism. Chemical oxidation by traces of ferric iron from the mineral may also have contributed to the formation of arsenate. The initial pH in inoculated flasks was 3.5. It dropped to 2.0 in 35 days. In contrast, in an uninoculated control, in which orpiment autoxidized but only slowly, the pH rose from 3.5 to 5, suggesting differences in the reactions in the experimental and control flasks. Realgar (AsS) was not attacked by *T. ferrooxidans* TM.

Arsenopyrite (FeAsS) and enargite (Cu_3AsS_4) were also oxidized by an iron-oxidizing *Thiobacillus* culture under the same test conditions as those used with orpiment (Ehrlich, 1964). During growth on arsenopyrite, the arsenic in the ore was transformed to arsenite and arsenate. The iron in the ore appeared ultimately as mobilized ferric iron. It precipitated extensively as ferric arsenite and arsenate. Because *T. ferrooxidans* does not oxidize arsenite to arsenate, the arsenate in the culture was probably formed through oxidation by ferric iron, although some autoxidation cannot be ruled out (Wakao et al., 1988; Braddock et al., 1984; Monroy-Fernandez et al., 1995). The pH dropped from 3.5 to 2.5 in both inoculated and uninoculated flasks in the first 21 days. However, in the uninoculated controls it rose to 4.3 by the 40th day while remaining at 2.5 in the inoculated flasks. Oxidation of arsenopyrite in the absence of the bacteria was significantly slower than in their presence (Fig. 13.2). In a later study, Carlson et

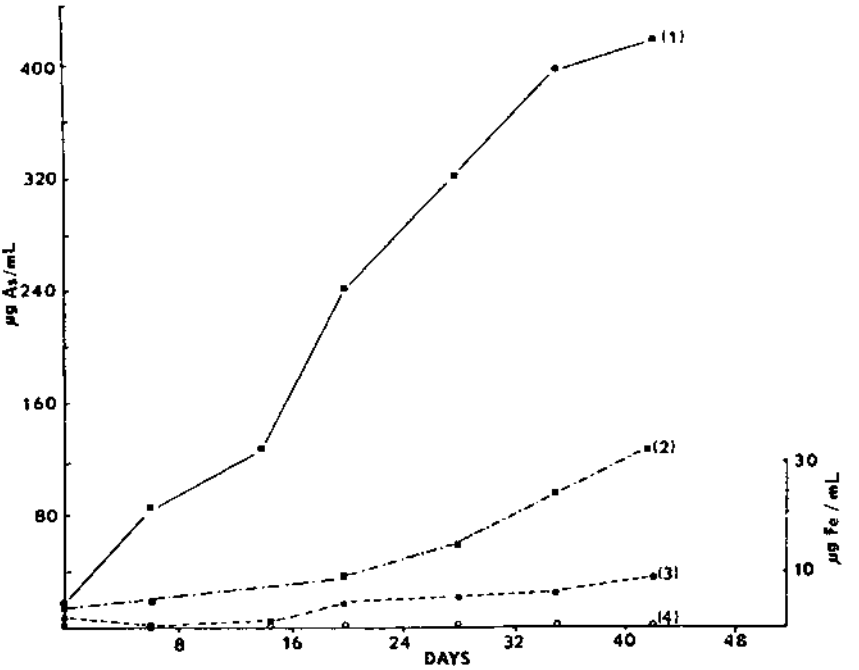


FIG. 13.1 Bacterial solubilization of orpiment. (1) Total arsenic released with bacteria (*T. ferrooxidans*); (2) total arsenic released without bacteria; (3) total iron released with bacteria (*T. ferrooxidans*); (4) total iron released without bacteria. (From Ehrlich, 1963, with permission.)

al. (1992) identified the minerals jarosite and scorodite among the solid products formed in the oxidation of arsenic-containing pyrite by a mixed culture of moderately thermophilic acidophiles. In another study with *T. ferrooxidans* at 22 °C and with a moderately thermophilic mixed culture at 45 °C on arsenopyrite, Tuovinen et al. (1994) found that the mixed culture oxidized the mineral nearly completely whereas *T. ferrooxidans* did not. Jarosite was a major sink for ferric iron in both cultures, but some amorphous ferric arsenate and S^0 were also formed. The absence of scorodite formation in this case was attributed to an insufficient drop in pH in the experiments. Groudeva et al. (1986) and Ngubane and Baecker (1990) reported that the extremely thermophilic archaeon *Sulfolobus* sp. oxidizes arsenopyrite.

Although *T. ferrooxidans* appears not to be able to oxidize arsenite to arsenate, the thermophilic archeon *Sulfolobus acidocaldarius* strain BC is able to do so (Sehlin and Lindström, 1992).

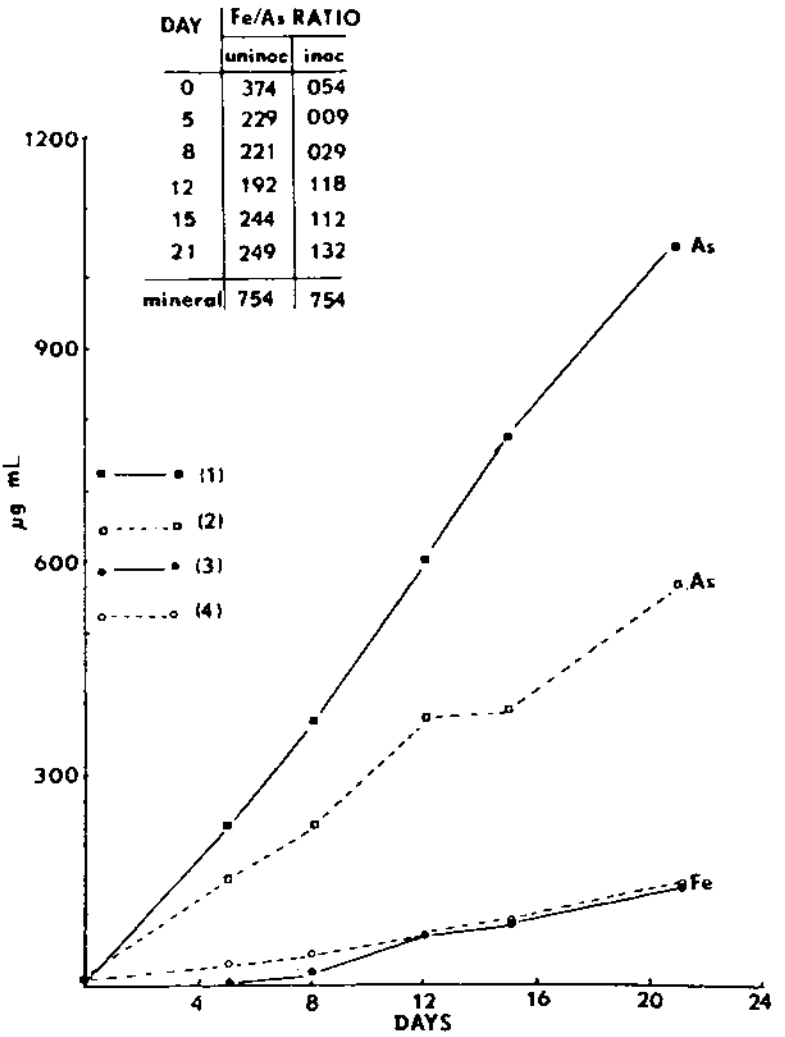


FIG. 13.2 Oxidation of arsenopyrite by *T. ferrooxidans*. Curves 1 and 3 represent changes in inoculated flasks. Curves 2 and 4 represent changes in uninoculated flasks. (From Ehrlich, 1964, with permission.)

Exposure of sterilized arsenopyrite particles for about 2 months in an underground area of the Richmond Mine at Iron Mountain, northern California (see Fig. 19.5), revealed very extensive surface attachment of rod-shaped bacteria, probably *Sulfobacillus* sp., accompanied by extensive pitting and the deposit of

elemental sulfur on the particle surface (Fig. 13.3). The S^0 represented more than 50% of the sulfur in the arsenopyrite.

Ehrlich (1964) found that a strain of *T. ferrooxidans*, when attacking enargite (Cu_3AsS_4), released cupric copper and arsenate into the bulk phase together with some iron that was present as an impurity in the mineral. The culture medium in which he performed the experiments contained (in $g\,L^{-1}$) $(NH_4)_2SO_4$, 3.0; KCl, 0.1; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $Ca(NO_3)_2$, 0.01, and was acidified to pH 3.5. To this salts solution, 0.5 g enargite ground to a mesh size of $+63$ μm was added. He found that in the presence of active bacteria, the pH of the reaction mixture dropped from 3.5 to between 2 and 2.5. In the absence of bacteria, the pH usually rose to 4.5. Some mobilized copper and arsenic precipitated in the experiments. The rate of enargite oxidation without bacteria was significantly slower and may have followed a different course of reaction on the basis of the different rates of Cu and As solubilization and the difference in pH changes.

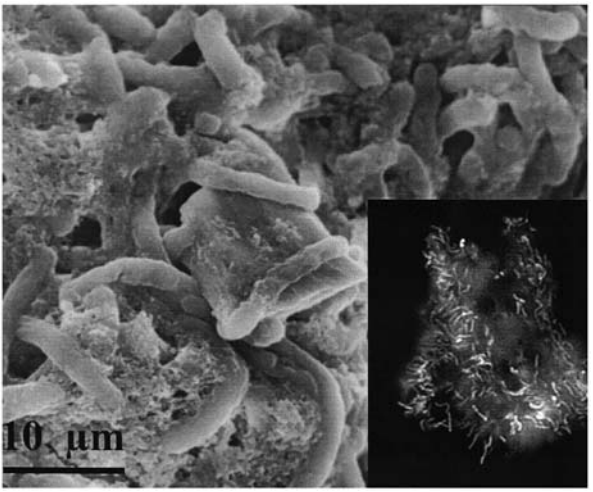


FIG. 13.3 Arsenopyrite particle with bacteria colonizing its surface. The particle was sterilized and then exposed for approximately 2 months (see Fig. 19.5) prior to examination by SEM. The image reveals a highly pitted surface covered with elongate cells and some secondary minerals. Based on microbial ecological studies at the site, the cells are probably *Sulfobacillus* sp. Insert shows an epifluorescence optical microscope image in which the cells on an arsenopyrite grain are labeled with a fluorescent dye that binds to DNA. Parallel experimental studies by Molly McGuire [see McGuire et al., 2001] demonstrated that $>50\%$ of the sulfur in the arsenopyrite ended up as elemental sulfur on the surface. (Images courtesy of Jill Banfield.)

Escobar et al. (1997) reported on the action of *T. ferrooxidans* strain ATCC 19859 on enargite, ground to a mesh size of $-147 +104\,\mu m$, and added to a salts solution containing (in $g\,L^{-1}$) $(NH_4)_2SO_4$, 0.4; $MgSO_4 \cdot 7H_2O$, 0.4; and KH_2PO_4, 0.056, acidified to pH 1.6. In the absence of added ferric iron, the mobilization of the copper in the enargite was more rapid and sustained in the presence of the bacteria than in the sterile medium. In the presence of ferric iron ($3.0\,g\,L^{-1}$), the mobilization of copper in enargite by the bacteria was more rapid and sustained than without added ferric iron. It was also more rapid than in sterile medium with ferric iron. Copper mobilization without bacteria but with added ferric iron was significantly faster than without ferric iron and without the bacteria but in both of these instances much less sustained than with the bacteria.

The ability of *T. ferrooxidans* and other acidophilic, iron-oxidizing bacteria to oxidize arsenopyrite is now being industrially exploited in beneficiation of sulfidic ores containing precious metals (Brierley and Brierley, 1999; Dew et al., 1997; Olson, 1994; Livesey-Goldblatt et al., 1983; Pol'kin et al., 1973; Pol'kin and Tanzhnyanskaya, 1968). The process involves the selective removal of arsenopyrite as well as pyrite by bacterial oxidation (bioleaching) before gold or silver is chemically (nonbiologically) extracted by cyanidation (complexing with cyanide). Arsenopyrite and pyrite interfere with cyanidation for several reasons. They encapsulate finely disseminated gold and silver, blocking access of cyanide reagent used to solubilize the gold and silver. They consume oxygen that is needed to convert metallic gold to aurous (Au^+) and auric (Au^{3+}) gold before it can be complexed by cyanide. Finally, the oxidation products of arsenopyrite and pyrite consume cyanide by forming thiocyanate and iron–cyanide complexes from which cyanide cannot be regenerated (Livesey-Goldblatt et al., 1983; Karavaiko et al., 1986). This increases the amount of cyanide reagent required for precious metal recovery.

Microbial removal of enargite from sulfidic gold ores is beginning to be considered as a beneficiation process in Chile (Acevedo et al., 1997; Escobar et al., 1997).

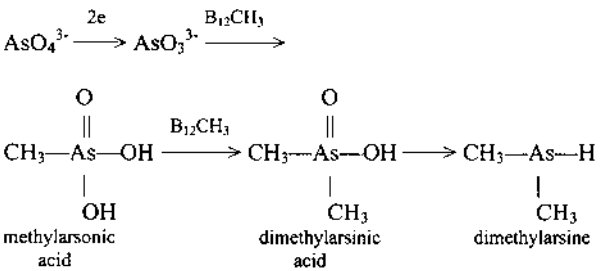
Beneficiation of carbonaceous gold ores containing as much as 6% arsenic by the action of *T. ferrooxidans* was reported by Kamalov et al. (1973). They succeeded in removing as much as 90% of the arsenic in the ore in 10 days under some circumstances. In another study, it was found possible to accelerate leaching of arsenic from a copper-tin-arsenic concentrate by 6–7-fold, using an adapted strain of *T. ferrooxidans* (Kulibakin and Laptev, 1971).

Microbial Reduction of Oxidized Arsenic Species

Some bacteria, fungi, and algae are able to reduce arsenic compounds. One of the first reports on arsenite reduction involved fungi (Gosio, 1897). Although

originally the product of this reduction was thought to be diethylarsine (Bignelli, 1901), it was later shown to be trimethylarsine (Challenger et al. 1933, 1951). A bacterium from cattle-dipping tanks that reduced arsenate to arsenite was also described early in the twentieth century (Green, 1918). Much more recently, it was reported that a strain of *Chlorella* was able to reduce a part of the arsenate it absorbs from the medium to arsenite (Blasco et al., 1971). Woolfolk and Whiteley (1962) demonstrated arsenate reduction to arsenite by H_2 with cell extracts of *Micrococcus* (*Veillonella*) *lactilyticus* and intact cells of *M. aerogenes*. The enzyme hydrogenase was involved. Arsine (AsH_3) was not formed in these reactions. *Pseudomonas* sp. and *Alcaligenes* sp. have been reported to able to reduce arsenate and arsenite anaerobically to arsine (Cheng and Focht, 1979).

Intact cells and cell extracts of the strict anaerobe *Methanobacterium* M.o.H. were shown to produce dimethylarsine from arsenate with H_2 (McBride and Wolfe, 1971). The extracts of this organism used methylcobalamine (CH_3B_{12}) as the methyl donor. The reaction required consumption of ATP. 5-CH_3-tetrahydrofolate or serine could not replace $B_{12}CH_3$, although CO_2 could when tested by isotopic tracer technique. The reaction sequence as described by McBride and Wolfe was

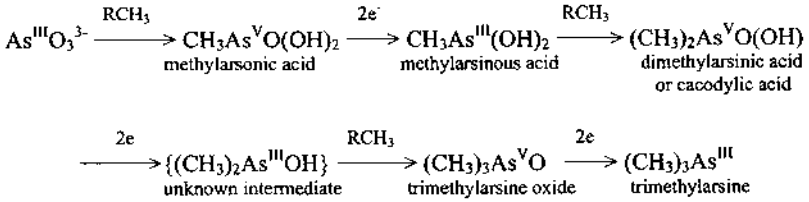


(13.2)

In an excess of arsenite, methylarsonic acid was the final product, the supply of CH_3B_{12} being limiting. In an excess of CH_3B_{12}, a second methylation step yielding dimethylarsinic acid (cacodylic acid) followed the first. The last step was shown to occur in the absence of CH_3B_{12}. All steps were enzymatic. Reaction sequence (13.2) was later found to be more complex (see below), and the natural methyl donor in *Methanobacterium* M.o.H (now *M. bryantii*) was shown to be methyl-coenzyme M (see discussion by Cullen and Reimer, 1989). Cell extracts of *Desulfovibrio vulgaris* were also found to produce a volatile arsinic derivative, presumably an arsine (McBride and Wolfe, 1971).

The fungus *Scopulariopsis brevicaulis* is able to convert arsenite to trimethylarsine. The reaction sequence originally proposed by Challenger

(1951) has been modified and includes the following steps (Cullen and Reimer, 1989, p. 720):



(13.3)

It should be noted that in this reaction sequence the oxidation state of the arsenic changes successively from +3 to +5 to +3. The reaction sequence leading to dimethylarsinic acid is similar to that observed in arsenic detoxification by methylation in human beings (Petrick et al., 2000; Aposhian et al., 2000). The methyl donors (RCH_3) in reaction (13.3) can be a form of methionine (see Cullen and Reimer, 1989). Besides *S. brevicaulis*, other fungi, such as *Aspergillus*, *Mucor*, *Fusarium*, *Paecilomyces*, and *Candida humicola*, have also been found active in such reductions (Alexander, 1977; Cox and Alexander, 1973; Pickett et al., 1981). Trimethylarsine oxide was found to be an intermediate in trimethylarsine formation by *C. humicola* (Pickett et al., 1981). The reduction of arsenate and arsenite to volatile arsines is a means of detoxification.

Arsenic Respiration

Among bacteria, some reduce arsenate only as far as arsenite. These organisms use the arsenate as terminal electron acceptor in anaerobic respiration. A number of them have been characterized (Table 13.2) (Stolz and Oremland, 1999; Oremland and Stolz, 2000). They include members of the domains Bacteria

TABLE 13.2 Arsenate-Respiring Bacteria and Archaea

Organism	Reference
Bacillus arsenicoselenatis	Switzer Blum et al., 1998
Bacillus selenitireducens	Switzer Blum et al., 1998; Oremland et al., 2000
Chrysiogenes arsenatis	Macy et al., 1996; Krafft and Macy, 1998
Desulfomicrobium strain Ben-RB	Macy et al., 2000
Desulfotomaculum auripigmentum	Newman et al., 1997a, 1997b
Pyrobaculum aerophilum[a]	Huber et al., 2000
Pyrobaculum arsenaticum[a]	Huber et al., 2000
Sulfurospirillum arsenophilum	Stolz et al., 1999
Sulfurospirillum barnesii	Laverman et al., 1995; Zobrist et al., 2000

[a] *Pyrobaculum* belongs to the domain of the Archaea.

and Archaea. Ahmann et al. (1994) isolated a comma-shaped motile rod, strain MIT-13, from arsenic-contaminated sediment from the Aberjona watershed in Massachusetts. This organism was later named *Sulfurospirillum arsenophilum*. It belongs to the Bacteria (Stolz et al., 1999). Anaerobically growing cultures of *S. arsenophilum* MIT-13 respired on arsenate using lactate but not acetate as electron donor and produced stoichiometric amounts of arsenite.

Chrysiogenes arsenatis, isolated from gold mine wastewater in Australia, is the only arsenate respirer isolated so far that is capable of using acetate as electron donor (Macy et al., 1996). Its arsenate reductase, a periplasmic enzyme, has been analyzed and found to consist of two subunits. One of these has a molecular mass of 87 kDa, and the other 29 kDA. They are assembled as a heterodimer with a native molecular mass of 123 kDa. The enzyme structure includes Zn, Fe, Mo, and acid-labile S (Krafft and Macy, 1998).

Sulfurospirillum barnesii strain SES-3 is another member of the Bacteria that can respire anaerobically on lactate using arsenate reduction to arsenite for terminal electron disposal (Laverman et al., 1995). Besides arsenate, it can also use selenate (see Chap. 20), Fe(III), thiosulfate, elemental sulfur, Mn(IV), nitrate, nitrite, trimethylamine *N*-oxide, and fumarate as terminal electron acceptors (Oremland et al., 1994). It can also grow microaerophilically, albeit sluggishly (15% O_2 optimum) (Laverman et al., 1995).

Newman et al. (1997a, 1997b) isolated *Desulfotomaculum auripigmentum* strain OREX-4 from sediment from Upper Mystic Lake, Massachusetts. It is a gram-positive rod belonging to the Bacteria. It anaerobically reduces arsenate to arsenite and sulfate to sulfide with lactate as electron donor. When both arsenate and sulfate are present together, the organism precipitates orpiment. The reactions can be summarized as follows (Newman et al., 1997b):

$$\begin{aligned}&CH_3CHOHCOO^- + 2HAsO_4^{2-} + 4H^+ \rightarrow \\ &CH_3COO^- + 2HAsO_2 + CO_2 + 3H_2O \\ &(\Delta G^{\circ\prime} = -41.1 \text{ kcal mol}^{-1} \text{ or } -172 \text{ kJ mol}^{-1} \text{ lactate} \qquad (13.4)\\ &CH_3CHOHCOO^- + 0.5SO_4^{2-} + 0.5H^+ \rightarrow \\ &CH_3COO^- + 0.5HS^- + CO_2 + H_2O \\ &(\Delta G^{\circ\prime} = -21.3 \text{ kcal mol}^{-1} \text{ or } -89 \text{ kJ mol}^{-1} \text{ lactate} \qquad (13.5)\\ &2HAsO_2 + 3HS^- + 3H^+ \rightarrow As_2S_3 + 4H_2O \qquad (13.6)\end{aligned}$$

Reactions (13.4) and (13.5) are catalyzed by the organism, whereas reaction (13.6) is not.

The microbial reduction of arsenate and sulfate when both are present together is sequential at rates that avoid the accumulation of excess sulfide. Orpiment did not form when arsenate and sulfide were added to the culture

medium in the absence of the bacteria. Maintenance of a pH of ~6.8 was essential for orpiment stability.

Macy et al. (2000) recently isolated two sulfate reducers belonging to the domain Bacteria that had the capacity to reduce arsenate to arsenite when lactate was used as the source of reductant. The lactate was oxidized to acetate. One of these organisms was *Desulfomicrobium* strain Ben-RB, which was able to reduce arsenate and sulfate concurrently and respired with arsenate as sole terminal electron acceptor in the absence of sulfate. The other strain was *Desulfovibrio* strain Ben-RA, which was also able to reduce arsenate and sulfate concurrently. However, it did not grow with arsenate as the sole terminal electron acceptor. In this organism, arsenate reduction may be part of an arsenate-resistance mechanism (see Silver, 1998).

Switzer Blum et al. (1998) discovered two species of the genus *Bacillus* in Mono Lake, California, that are able to reduce not only arsenate but also selenium oxyanions (see Chap. 20). They are *Bacillus arsenicoselenatis* and *B. selenitireducens* and belong to the domain Bacteria. Both organisms are moderate halophiles and alkaliphiles, growing maximally between pH 9 and 11. Each reduces arsenate to arsenite.

Huber et al. (2000) isolated a member of the domain Archaea, *Pyrobaculum arsenaticum*, from a hot spring at Pisciarelli Solfataras at Naples, Italy, capable of reducing arsenate as terminal electron acceptor using H_2 as electron donor. The organism is a hyperthermophilic, facultative autotroph that reduces arsenate to arsenite in its anaerobic respiration. In addition to arsenate, it can use thiosulfate and elemental sulfur as alternative electron acceptors for autotrophic growth. Organotrophically, the organism produces realgar (As_2S_2) when using arsenate plus thiosulfate or arsenate plus cysteine as terminal electron acceptors. It can also reduce selenate but not selenite organotrophically to elemental selenium in a respiratory process.

Another species of *Pyrobaculum*, *P. aerophilum*, can reduce arsenate and selenate as terminal electron acceptors in autotrophic growth on H_2 as electron donor (Huber et al., 2000). Organotrophically, this organism can use arsenate as terminal electron acceptor when growing anaerobically. However, it does not form a visible precipitate of realgar on arsenate plus thiosulfate or arsenate plus cysteine. Selenate and selenite can be alternative electron acceptors. The selenium compounds are reduced to elemental selenium.

Direct Observations of Arsenite Oxidation and Arsenate Reduction In Situ

Oxidation of arsenite to arsenate in activated sludge was reported by Myers et al. (1973). Arsenate was reduced stepwise to lower oxidation states over an extended period in the activated sludge. *P. fluorescens* was an active reducer in this system

under overall aerobic conditions (Myers et al., 1973). Reduction may actually have occurred in anaerobic microenvironments in the floc.

Oremland et al. (2000) developed extensive evidence of arsenate reduction in meromictic, alkaline, hypersaline Mono Lake, California, which contains approximately 200 μM total arsenic. The oxidation state of the dissolved arsenic changed from predominantly +5 in the oxic surface waters (mixolimnion) to +3 in the anoxic bottom waters (monimolimnion). No significant bacterial reduction was noted in the oxic waters, but it was significant in the anoxic waters. A rate of $\sim 5.9\ \mu mol\ L^{-1}\ day^{-1}$ was measured at depths between 18 and 21 m. Sulfate reduction occurred at depths below 21 m, the highest rate of $\sim 2.3\ \mu mol\ L^{-1}\ day^{-1}$ being measured at a depth of 28 m. Oremland et al. (2000) estimated that arsenate respiration is second in importance after sulfate respiration in Mono Lake and may account for mineralization of 14.2% annually of the photosynthetically fixed carbon in the lake.

Johnson (1972) made direct observations relating to microbial reduction of arsenic in the ocean. He found that bacteria from phytoplankton samples in Narragansett Bay and from Sargasso Sea water were able to reduce added arsenate. An arsenate reduction rate of $10^{-11}\ \mu mol\ cell^{-1}\ min^{-1}$ was measured over 12 hr period of incubation at 20–22°C. Arsenic was not accumulated by the bacteria, and none was lost from the medium through volatilization. These observations may help to explain why the observed As^{+5}/As^{+3} ratio in the seawater was $10^{-1}:10$ instead of $10^{26}:1$, as predicted under equilibrium conditions in oxygenated seawater at pH 8.1 (Johnson, 1972; Andreae, 1979).

Cheng and Focht (1979) observed that bacteria can reduce arsenate and arsenite to arsine under anaerobic conditions in soils. They indicated that, unlike fungi, the bacteria produce mono- and dimethylarsine only when methylarsonate or dimethylarsinate is available.

Figure 13.4 summarizes the reactions involving arsenic compounds in nature that are catalyzed by microorganisms. The oxidation of methylated arsine, although not indicated in the diagram, has been suggested by Cheng and Focht (1979). The arsenic cycle in natural freshwaters has been discussed by Ferguson and Gavis (1972) and Scudlark and Johnson (1982).

13.3 ANTIMONY

Antimony Distribution in the Earth's Crust

Antimony is a rare element. Its abundance in the Earth's crust when normalized to its abundances in chondritic stone meteorites and adjusted to Si = 1.00 has been given as 0.1 (Winchester, 1972). Its average concentration in igneous rocks is 0.2 ppm, in shales 1.5 ppm, in limestone 0.3 ppm, in sandstone 0.05 ppm, and in soil 1 ppm. Its average concentration in seawater is 0.24 ppb and in freshwater

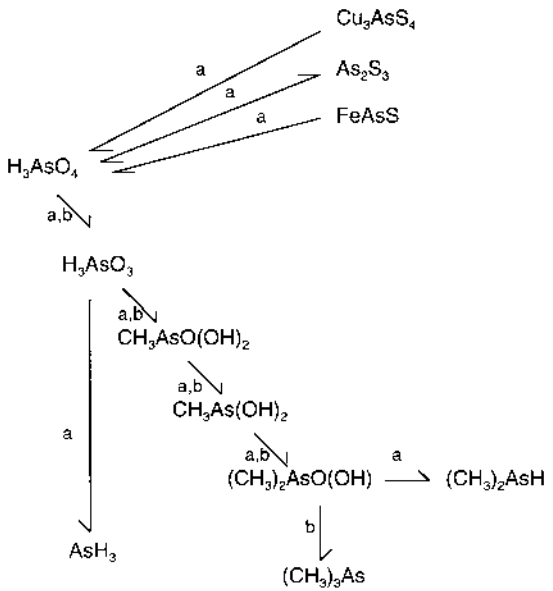


FIG. 13.4 Summary of possible microbial interactions with arsenic compounds (a) performed by bacteria, (b) performed by fungi.

0.2 ppb (Bowen, 1979). As a mineral, it may occur as stibnite (Sb_2S_3), kermesite (Sb_2S_2O), senarmontite (Sb_2O_3), jamesonite ($2PbS \cdot Sb_2S_3$), boulangerite ($5PbS{\cdot}2Sb_2S_3$), and tetrahedrite (Cu_3SbS_3). It may also occur as sulfantimonides of copper, silver, and nickel, and sometimes as elemental antimony (Gornitz, 1972). Stibnite is the most common antimony mineral. Antimony can exist in the oxidation states -3, 0, $+3$ and $+5$. Like arsenic compounds, antimony compounds tend to be toxic to most living organisms. The basis for this toxicity has not been clearly established.

Microbial Oxidation of Antimony Compounds

Among the earliest reports of the bio-oxidation of antimony-containing minerals is one by Bryner et al. (1954) in which the oxidation of tetrahedrite ($4Cu_2S \cdot Sb_2S_3$) by *Thiobacillus ferrooxidans* is recorded. Lyalikova (1961) reported the oxidation of antimony trisulfide (Sb_2S_3) by *T. ferrooxidans*. In both these instances, the oxidation proceeded under acid conditions (pH 2.45) (Kuznetsov et al., 1963). The oxidation state of the solubilized antimony was not determined in either case. More recently, Silver and Torma (1974) reported on the oxidation of synthetic antimony sulfides by *T. ferrooxidans*. Torma and Gabra

(1977) examined the oxidation of stibnite (Sb_2S_3) by the same organism. The latter authors suspected that *T. ferrooxidans* oxidized trivalent antimony [Sb(III)] to pentavalent [Sb(V)] but offered no proof. Lyalikova et al. (1972) reported on the oxidation of Sb-Pb sulfides, Sb-Pb-Te sulfides, and Sb-Pb-As sulfides resulting in the formation of such minerals as anglesite ($PbSO_4$) and valentinite (Sb_2O_3). The formation of valentinite suggests that antimony in the minerals was solubilized but not oxidized. Ehrlich (1986, 1988) reported on the oxidation by *T. ferrooxidans* strain 19759 of a mixed sulfide ore that included tetrahedrite. Although he followed the mobilization of silver, copper, and zinc from the ore in these experiments, he did not follow the mobilization of antimony. In all the foregoing studies, most or all of the oxidation apparently involved the sulfide in the minerals.

Trivalent antimony is susceptible to direct oxidation by *Stibiobacter senarmontii* (Lyalikova, 1972, 1974; Lyalikova et al., 1976). This organism, which was isolated from a sample of Yugoslavian mine drainage, oxidizes senarmontite (Sb_2O_3) or Sb_2O_4 to Sb_2O_5, deriving useful energy from the process. It is a gram-positive rod ($0.5–1.8 \times 0.5\ \mu m$) with a single polar flagellum and has the ability to form rudimentary mycelia in certain stages of development. It grows at neutral pH and generates acid when oxidizing Sb_2O_3 (the pH can drop from 7.5 to 5.5). When grown on reduced antimony oxide (senarmontite), the organism possesses the enzyme ribulose bisphosphate carboxylase, indicating that it has chemolithotrophic propensity (Lyalikova et al., 1976). Antimony sulfide ores can thus be oxidized to antimony pentoxide in two steps:

$$Sb_2S_3 \xrightarrow[(1)]{O_2} Sb_2O_3 \xrightarrow[(2)]{O_2} Sb_2O_5 \tag{13.7}$$

The first step is catalyzed by an organism such as *Thiobacillus* Y or *T. ferrooxidans* [see (1) in reaction (13.7)] and the second by *Stibiobacter senarmontii* [see (2) in reaction (13.7)] (Lyalikova et al., 1974).

Microbial Reduction of Oxidized Antimony Minerals

No evidence exists that microbes are able to reduce oxidized antimony compounds, whether as terminal electron acceptor in anaerobic respiration or for other purposes (see, e.g., Iverson and Brinckman, 1978). This lack of evidence should not, however, be taken to mean that microbial antimony reduction does not occur, but rather that it has so far not been observed.

13.4 SUMMARY

Although arsenic and antimony compounds are toxic to most forms of life, some microbes metabolize them. Arsenite has been found to be enzymatically oxidized

by several different bacteria. The enzyme system is inducible. In laboratory experiments, *Alcaligenes faecalis* oxidized arsenite most intensely only after having gone through active growth. The organism probably can derive maintenance energy from arsenite oxidation. *Pseudomonas arsenitoxidans* and two as yet unnamed isolates from Australia can grow autotrophically with arsenite as their sole source of energy. Simple and complex arsenic sulfides are oxidized by *Thiobacillus ferrooxidans*. No evidence has been obtained, however, that this organism can oxidize trivalent to pentavalent arsenic enzymatically.

Arsenate and arsenite have been shown to be reduced by certain bacteria and fungi. When used as terminal electron acceptor, arsenate is reduced to arsenite. In arsenic detoxification, bacteria can reduce arsenate or arsenite to arsine or dimethylarsine, whereas fungi produce trimethylarsine. All of these arsines are volatile.

Antimony compounds have also been shown to be microbially oxidized. *T. ferrooxidans* has been shown to attack a variety of antimony-containing sulfides. Although enzymatic oxidation by *T. ferrooxidans* of Sb(III) to Sb(V) has been claimed in the case of Sb_2S_3, clear proof is lacking. Generally, only the sulfide moiety, and ferrous iron if present, in an antimony mineral are oxidized by this organism. *Stibiobacter senarmontii*, an autotroph that was isolated from an ore deposit in Yugoslavia, does oxidize the trivalent antimony in Sb_2O_3 or Sb_2O_4 to pentavalent antimony (Sb_2O_5). Microbial reduction of oxidized antimony compounds has not been reported so far.

REFERENCES

Abdrashitova SA, Mynbaeva BN, Ilyaletdinov AN. 1981. Oxidation of arsenic by the heterotrophic bacteria *Pseudomonas putida* and *Alcaligenes eutrophus*. Mikrobiologiya 50:41–45 (Engl transl pp 28–31).

Acevedo F, Gentina JC, Alegre C, Arévalo P. 1997. Biooxidation of a gold bearing enargite concentrate. In: International Biohydrometallurgy Symposium IBS97 Biomine 97, "Biotechnology Comes of Age." Conf Proc. Glenside, South Australia: Australian Mineral Foundation, pp M3.2.1–M3.2.9.

Ahmann D, Roberts AL, Krumholz LR, Morel FMM. 1994. Microbe grows by reducing arsenic. Nature (Lond) 371:750.

Alexander M. 1977. Introduction to Soil Microbiology. New York: Wiley.

Anderson GL, Williams J, Hille R. 1992. The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. J Biol Chem 267:23674–23682.

Andreae MO. 1979. Arsenic speciation in seawater and interstitial waters: The influence of biological-chemical interactions on the chemistry of a trace element. Limnol Oceanogr 24:440–452.

Aposhian HV, Zheng B, Aposhian MM, Le XC, Cebrian ME, Cullen W, Zakharian RA, Ma M, Dart RC, Cheng Z, Andrewes P, Yip L, O'Malley GF, Maiorino RM, Van

Voorhies W, Healy SM, Titcomb A. 2000. DMPS: Arsenic challenge test. II. Modulation of arsenic species, including monomethylarsonous acid (MMA^{III}), excreted in human urine. Toxicol Appl Pharm 165:74–83.

Bennett RL, Malamy MH. 1970. Arsenate-resistant mutants of *Escherichia coli* and phosphate transport. Biochem Biophys Res Commun 40:496–503.

Bignelli P. 1901. Gazz Chim Ital 31:58 (as cited by Challenger, 1951).

Blasco F, Gaudin C, Jeanjean R. 1971. Absorption of arsenate ions by *Chlorella*. Partial reduction of arsenate to arsenite. CR Acad Sci (Paris) Ser D 273:812–815.

Bowen HJM. 1979. Environmental Chemistry of the Elements. London: Academic Press.

Braddock JF, Luong, HV, Brown EJ. 1984. Growth kinetics of *Thiobacillus ferrooxidans* isolated from mine drainage. Appl Environ Microbiol 48:48–55.

Brierley JA, Brierley CL. 1999. Present and future commercial applications of biohydrometallurgy. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century. Part A. Amsterdam: Elsevier, pp 81–89.

Broeer S, Ji G, Broeer A, Silver S. 1993. Arsenic efflux governed by the arsenic resistance plasmid pI258. J Bacteriol 175:3480–3485.

Bryner LC, Beck JV, Davis BB, Wilson DG. 1954. Microorganisms in leaching sulfide minerals. Ind Eng Chem 46:2587–2592.

Buchanan RE, Gibbons NE. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore, MD: Williams & Wilkins.

Button DK, Dunker SS, Moore ML. 1973. Continuous culture of *Rhodotorula rubra*: Kinetics of phosphate-arsenate uptake, inhibition and phosphate-limited growth. J Bacteriol 113:599–611.

Carapella SC Jr. 1972. Arsenic: Element and geochemistry. In: Fairbridge RW, ed. The Encylopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci, Ser IVA. New York: Van Nostrand Reinhold, pp 41–42.

Carlson L, Lindström EB, Hallberg KB, Tuovinen OH. 1992. Solid-phase production of bacterial oxidation of arsenic/pyrite. Appl Environ Microbiol 58:1046–1049.

Challenger F. 1933. The formation of organo-metalloidal compounds by microorganisms. Part II. Trimethylarsine and dimethylarsine. J Chem Soc 1933:95–101.

Challenger F. 1951. Biological methylation. Adv Enzymol 12:429–491.

Cheng C-N, Focht DD. 1979. Production of arsine and methylarsines in soil and in culture. Appl Environ Microbiol 38:494–498.

Cox DP, Alexander M. 1973. Effect of phosphate and other anions on trimethylarsine formation by *Candida humicola*. Appl Environ Microbiol 25:408–413.

Cullen WR, Reimer KJ. 1989. Arsenic speciation in the environment. Chem Rev 89:713–784.

Da Costa EWB. 1971. Suppression of the inhibitory effects of arsenic compounds by phosphate. Nature (New Biol) (Lond) 231:32.

Da Costa EWB. 1972. Variation in the toxicity of arsenic compounds to microorganisms and the suppression of the inhibitory effects of phosphate. Appl Environ Microbiol 23:46–53.

Dew DW, Lawson EN, Broadhurst JL. 1997. The BIOX® process for biooxidation of gold-bearing ores and concentrates. In: Rawlings DE, ed. Biomining. Theory, Microbes and Industrial Processes. Berlin, Germany: Springer, pp 45–80.

Dyke KGH, Parker MT, Richmond MH. 1970. Penicillinase production and metal-ion resistance in *Staphylococcus aureus* cultures isolated from hospital patients. J Med Microbiol 3:125–136.

Ehrlich HL. 1963. Bacterial action on orpiment. Econ Geol 58:991–994.

Ehrlich HL. 1964. Bacterial oxidation of arsenopyrite and enargite. Econ Geol 59:1306–1312.

Ehrlich HL. 1978. Inorganic energy sources for chemolithotrophic and mixotrophic bacteria. Geomicrobiol J 1:65–83.

Ehrlich HL. 1986. Bacterial leaching of silver from silver-containing mixed sulfide ore by a continuous process. In: Lawrence RW, Branion RMR, Ebner HG, eds. Fundamental and Applied Biohydrometallurgy. Amsterdam: Elsevier, pp 77–88.

Ehrlich HL. 1988. Bioleaching of silver from a mixed sulfide ore in a stirred reactor. In: Norris PR, Kelly DP, eds. Biohydrometallurgy. Kew, Surrey, UK: Science and Technology Letters, pp 223–231.

Escobar B, Huenupi E, Wiertz J. 1997. Chemical and biological leaching of enargite. Biotechnol Lett 19:719–722.

Ferguson JF, Gavis J. 1972. A review of the arsenic cycle in natural waters. Water Res 6:1259–1274.

Gornitz V. 1972. Antimony: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci, Ser IVA. New York: Van Nostrand Reinhold, pp 33–36.

Gosio B. 1897. Ber (Deut Chem Ges) 30:1024 (as cited by Challenger, 1951).

Green HH. 1918. Description of a bacterium which oxidizes arsenite to arsenate, and of one which reduces arsenate to arsenite, isolated from a cattle-dipping tank. S Afr J Sci 14:465–467.

Groudeva VI, Groudev SN, Markov KI. 1986. A comparison between mesophilic and thermophilic bacteria with respect to their ability to leach sulfide minerals. In: Lawrence RW, Branion RMR, Ebner HG, eds. Fundamental and Applied Biohydrometallurgy. Amsterdam: Elsevier, pp 484–485.

Hedges RW, Baumberg S. 1973. Resistance to arsenic compounds conferred by a plasmid transmissible between strains of *Escherichia coli*. J Bacteriol 115:459–460.

Hendrie MS, Holding AJ, Shewan JM. 1974. Emended description of the genus *Alkaligenes* and of *Alkaligenes faecalis* and a proposal that the generic name of *Achromobacter* be rejected: Status of the named species of *Alkaligenes* and *Achromobacter*. Int J Syst Bacteriol 24:534–550.

Huber R, Sacher M, Vollman A, Huber H, Rose D. 2000. Respiration of arsenate and selenate by hyperthermophilic Archaea. Syst Appl Microbiol 23:305–314.

Ilyaletdinov AN, Abdrashitova SA. 1981. Autotrophic oxidation of arsenic by a culture of *Pseudomonas arsenitoxidans*. Mikrobiologiya 50:197–204 (Engl transl pp 135–140).

Iverson WP, Brinckman FE. 1978. Microbial metabolism of heavy elements. In: Mitchell R, ed. Water Pollution Microbiology, Vol 2. New York: Wiley, pp 201–232.

Ji G, Silver S. 1992. Regulation and expression of the arsenic resistance operon from *Staphylococcus aureus* plasmid pI258. J Bacteriol 174:3684–3694.

Ji G, Silver S, Garber EAE, Ohtake H, Cervantes C, Corbisier P. 1993. Bacterial molecular genetics and enzymatic transformations of arsenate, arsenite, and chromate. In: Torma

AE, Apel ML, Brierley CL, eds. Biohydrometallurgical Technologies, Vol 2. Fossil Energy Materials Bioremediation, Microbial Physiology. Warrendale, PA: Minerals, Metals, Mater Soc, pp 529–539.

Johnson DL. 1972. Bacterial reduction of arsenite in seawater. Nature (Lond) 240:44–45.

Kamalov MR, Karavaiko GI, Ilyaletdinov AN, Abrashitova SA. 1973. The role of *Thiobacillus ferrooxidans* in leaching arsenic from a concentrate of carbonaceous gold-containing ore. Izv Akad Nauk Kaz SSR Ser Biol 11:37–44.

Karavaiko GI, Chuchalin LK, Pivovarova TA, Yemel'yanov BA, Forofeyev AG. 1986. Microbiological leaching of metals from arsenopyrite containing concentrates. In: Lawrence RW, Branion RMR, Ebner HG, eds. Fundamental and Applied Biohydrometallurgy. Amsterdam: Elsevier, pp 125–126.

Krafft T, Macy JM. 1998. Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. Eur J Biochem 255:647–653.

Kulibakin VG, Laptev SF. 1971. Effect of adaptation of *Thiobacillus ferrooxidans* to a copper-arsenic-tin concentrate on the arsenic leach rate. Sb Tr Tsent Nauk-Issled Inst Olomyan Prom 1:75–76.

Kuznetsov SI, Ivanov MV, Lyalikova NN. 1963. Introduction to Geological Microbiology. Engl transl. New York: McGraw-Hill.

Laverman AM, Switzer Blum J, Schaefer JK, Phillips EJP, Lovley DR, Oremland RS. 1995. Growth of strain SES-3 with arsenate and other diverse electron acceptors. Appl Environ Microbiol 61:3556–3561.

Legge JW. 1954. Bacterial oxidation of arsenite. IV. Some properties of the bacterial cytochromes. Aust J Biol Sci 7:504–514.

Legge JW, Turner AW. 1954. Bacterial oxidation of arsenite. III. Cell-free arsenite dehydrogenase. Aust J Biol Sci 7:496–503.

Livesey-Goldblatt E, Norman P, Livesey-Goldblatt DR. 1983. Gold recovery from arsenopyrite/pyrite ore by bacterial leaching and cyanidation. In: Rossi G, Torma AE, eds. Recent Progress in Biohydrometallurgy. Iglesias, Italy: Assoc Mineraria Sarda, pp 627–641.

Lunde G. 1973. Synthesis of fat and water soluble arseno organic compounds in marine and limnetic algae. Acta Chem Scand 27:1586–1594.

Lyalikova NN. 1961. The role of bacteria in the oxidation of sulfide ores. Tr In-ta Mikrobiol AN SSSR, No. 9 (as cited by Kuznetsov et al., 1963).

Lyalikova NN. 1972. Oxidation of trivalent antimony to higher oxides as an energy source for the development of a new autotrophic organism *Stibiobacter* gen. n. Dokl Akad Nauk SSSR Ser Biol 205:1228–1229.

Lyalikova NN. 1974. *Stibiobacter senarmontii*: A new antimony-oxidizing microorganism. Mikrobiologiya 43:941–948 (Engl transl pp 799–805).

Lyalikova NN, Shlain LB, Unanova OG, Anisimova LS. 1972. Transformation of products of compound antimony and lead sulfides under the effect of bacteria. Izv Akad Nauk SSSR Ser Biol 4:564–567.

Lyalikova NN, Shlain LB, Trofimov VG. 1974. Formation of minerals of antimony(V) under the effect of bacteria. Izv Akad Nauk SSSR Ser Biol 3:440–444.

Lyalikova NN, Vedenina IYa, Romanova AK. 1976. Assimilation of carbon dioxide by a culture of *Stibiobacter senarmontii*. Mikrobiologiya 45:552–554 (Engl transl pp 476–477).

Macy JM, Nunan K, Hagen KD, Dixon DR, Harbour PJ, Cahill M, Sly L. 1996. *Chrysiogenes arsenatis* gen. nov., spec. nov., a new arsenate-respiring bacterium isolated from gold mine wastewater. Int J Syst Bacteriol 46:1153–1157.

Macy JM, Santini, JM, Pauling BV, O'Neill AH, Sly LI. 2000. Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Arch Microbiol 173:49–57.

Mahler HR, Cordes EH. 1966. Biological Chemistry. New York: Harper and Row.

McBride BS, Wolfe RS. 1971. Biosynthesis of dimethylarsine by *Methanobacterium*. Biochemistry 10:4312–4317.

McGuire MM, Banfield JF, Hamers RJ. 2001. Quantitative Determination of Elemental Sulfur at the Arsenopyrite Surface After Oxidation by Ferric Iron: Mechanistic Implications. Geochemical Transactions Vol. 4; Digital Object Indentifier: 10.1039/b104111h.

Monroy-Fernández MG, Mustin C, de Donato P, Berthelin J, Barion P. 1995. Bacterial behavior and evolution of surface oxidized phases during arsenopyrite oxidation by *Thiobacillus ferrooxidans*. In: Vargas T, Jerez JV, Toledo H, eds. Biohydrometallurgical Processing. Vol 1. Santiago: Univ Chile, pp 57–66.

Myers DJ, Heimbrook ME, Osteryoung J, Morrison SM. 1973. Arsine oxidation state in the presence of microorganisms. Examination by differential pulse polarography. Environ Lett 5:53–61.

Newman DK, Beveridge TJ, Morel FM. 1997a. Precipitation of arsenic trisulfide by *Desulfotomaculum auripigmentum*. Appl Environ Microbiol 63:2022–2028.

Newman DK, Kennedy EK, Coates JD, Ahmann D, Ellis DJ, Lovley DR, Morel FM. 1997b. Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum*, sp. nov. Arch Microbiol 168:380–388.

Ngubane WT, Baecker AAW. 1990. Oxidation of gold-bearing pyrite and arsenopyrite by *Sulfolobus acidocaldarius* and *Sulfolobus* BC in airlift bioreactors. Biorecovery 1:225–269.

Olson GJ. 1994. Microbiological oxidation of gold ores and gold bioleaching. FEMS Microbiol Lett 119:1–6.

Oremland RS, Stolz J. 2000. Dissimilatory reduction of selenate and arsenate in nature. In: Lovley DR, ed. Environmental Microbial-Metal Interactions. Washington, DC: ASM Press, pp 199–224.

Oremland RS, Switzer Blum J, Culbertson CW, Visscher PT, Miller LG, Dowdle P, Strohmaier FE. 1994. Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Appl Environ Microbiol 60:3011–3019.

Oremland RS, Dowdle PR, Hoeft S, Sharp JO, Schaefer JK,Miller LG, Switzer Blum J, Smith RL, Bloom NS, Wallschlaeger D. 2000. Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. Geochim Cosmochim Acta 64:3073–3084.

Osborne FH. 1973. Arsenite oxidation by a soil isolate of *Alcaligenes*. PhD Thesis. Rensselaer Polytechnic Inst, Troy, NY.

Osborne FH, Ehrlich HL. 1976. Oxidation of arsenite by a soil isolate of *Alcaligenes*. J Appl Bacteriol 41:295–305.

Oscarson DW, Huang PM, Defosse C, Herbillon A. 1981. Oxidative power of Mn(IV) and Fe(III) oxides with respect to As(III) in terrestrial and aquatic environments. Nature (Lond) 291:50–51.

Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Aposhian HV. 2000. Monomethylarsonous acid (MMA^{III}) is more toxic than arsenite in Chang human erythrocytes. Toxicol Appl Pharmacol 163:203–207.

Phillips SE, Taylor ML. 1976. Oxidation of arsenite and arsenate by *Alkaligenes faecalis*. Appl Environ Microbiol 32:392–399.

Pickett AW, McBride BC, Cullen WR, Manji H. 1981. The reduction of trimethylarsine oxide by *Candida humicola*. Can J Microbiol 27:773–778.

Pol'kin SI, Tanzhnyanskaya ZA. 1968. Use of bacterial leaching for ore enrichment. Izv Vyssch Ucheb Zaved Tsvet Met 11:115–121.

Pol'kin SI, Yudina N, Nanin VV, Kim DKh. 1973. Bacterial leaching of arsenic from an arsenopyrite gold-containing concentrate in a thick pulp. Nauchno-Issled Geologorazved Inst Tsvetn Blagorodn Metall 107:34–41.

Quastel JH, Scholefield PG. 1953. Arsenite oxidation in soil. Soil 75:279–285.

Santini JM, Sly LI, Schnagl RD, Macy JM. 2000. A new chemolithotrophic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. Appl Environ Microbiol 66:92–97.

Scudlark JR, Johnson DL. 1982. Biological oxidation of arsenite in seawater. Estuarine Coastal Shelf Sci 14:693–706.

Sehlin HM, Lindström EB. 1992. Oxidation and reduction of arsenic by *Sulfolobus acidocaldarius* strain BC. FEMS Microbiol Lett 93:87–92.

Silver M, Torma AE. 1974. Oxidation of metal sulfides by *Thiobacillus ferrooxidans* grown on different substrates. Can J Microbiol 20:141–147.

Silver S. 1998. Genes for all metals: A bacterial view of the periodic table. J Ind Microbiol Biotechnol 180:3686–3691.

Silver S, Keach D. 1982. Energy-dependent arsenate flux: The mechanism of plasmid-mediated resistance. Proc Natl Acad Sci USA 79:6114–6118.

Silverman MP, Lundgren DG. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. J Bacteriol 77:642–647.

Stolz JF, Oremland RS. 1999. Bacterial respiration of arsenic and selenium. FEMS Microbiol Rev 23:615–627.

Stolz JF, Ellis DJ, Switzer Blum J, Ahmann D, Lovley DR, Oremland RS. 1999. *Sulfurospirillum barnesii* sp. nov., *Sulfurospirillum arsenophilus* sp. nov., and the *Sulfurospirillum* clade in the Epsilon Proteobacteria. Int J Syst Bacteriol 49: 1177–1180.

Switzer Blum J, Burns Bindi A, Buzzelli J, Stolz JF, Oremland RS. 1998. *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: Two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30.

Torma AE, Gabra GG. 1977. Oxidation of stibnite by *Thiobacillus ferrooxidans*. Antonie v Leeuwenhoek 43:1–6.

Tuovinen OH, Bhatti TM, Bigham JM, Hallberg KB, Garcia O Jr, Lindström EB. 1994. Oxidative dissolution of arsenopyrite by mesophilic and moderately thermophilic acidophiles. Appl Environ Microbiol 60:3268–3274.

Turner AW. 1949. Bacterial oxidation of arsenite. Nature (Lond) 164:76–77.

Turner AW. 1954. Bacterial oxidation of arsenite. I. Description of bacteria isolated from arsenical cattle-dipping fluids. Aust J Biol Sci 7:452–478.

Turner AW, Legge JW. 1954. Bacterial oxidation of arsenite. II. The activity of washed suspensions. Aust J Biol Sci 7:479–495.

Wakao N, Koyatsu H, Komai Y, Shimokawara H, Sakurai Y, Shibota H. 1988. Microbial oxidation of arsenite and occurrence of arsenite-oxidizing bacteria in acid mine water from a sulfur-pyrite mine. Geomicrobiol J 6:11–24.

Weast RC, Astle MJ. 1982. CRC Handbook of Chemistry and Physics. 63rd ed. Boca Raton, FL: CRC Press.

Weeger W, Lièvremont D, Perret M, Lagarde F, Hubert J-C, Leroy M, Lett M-C. 1999. Oxidation of arsenite to arsenate by a bacterium from an aquatic environment. BioMetals 12:141–149.

Winchester JW. 1972. Geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 402–410.

Woolfolk CA, Whiteley HR. 1962. Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. I. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements. J Bacteriol 84:647–658.

Zobrist J, Dowdle PR, Davis JA, Oremland RS. 2000. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. Environ Sci Technol 34:4747–4753.

14

Geomicrobiology of Mercury

14.1 INTRODUCTION

The element mercury has been known as a specific chemical from at least as far back as 1500 B.C. The physician Paracelsus (A.D. 1493–1541) attempted to cure syphilis by administering metallic mercury to sufferers of the disease. His treatment was probably based on intuitive or empirical knowledge that at an appropriate dosage, mercury was more toxic to the cause of the disease than to the patient. The true etiology of syphilis was, however, unknown to him. The extent of mercury toxicity for human beings and other animals became very apparent only in recent times as a consequence of environmental pollution by mercury compounds. The toxicity manifests itself in major physical impairments and death due to intake of the compounds in food and water. Incidents of mercury poisoning in Japan (Minimata disease), Iraq, Pakistan, Guatemala, and the United States drew special attention to the problem. In some of these cases, food was consumed that had been made from seed grain treated with mercury compounds to inhibit fungal damage before planting. The seed grain had not been intended for food use. In other cases, food such as meat had become tainted because the animals yielding the meat drank water that had become polluted by mercury compounds or they had eaten mercury-tainted feed. Tracing the fate of mercury introduced into the environment has revealed an intimate role of microbes in the interconversion of some mercury compounds.

14.2 DISTRIBUTION OF MERCURY IN THE EARTH'S CRUST

The abundance of mercury in the Earth's crust has been reported as 0.08 ppm (Jonasson, 1970). Its concentration in uncontaminated soils has been given as 0.007 ppm (Jonasson, 1970). Its concentration in freshwaters may range from 0.01 to 10 ppb, although concentrations as high as 1600 ppb have been measured in waters in contact with copper deposits in the southern Urals (Jonasson, 1970). The maximum permissible level in potable waters was set at 1 ppb as of 1990 by the World Health Organization (WHO) (Cotruvo and Vogt, 1990). The average mercury concentration in seawater has been reported as 0.2 ppb (Marine Chemistry, 1971).

In nature, mercury can exist as metal or in inorganic and organic compounds. The metal is liquid at ambient temperature and has a significant vapor pressure (1.2×10^{-3} mmHg at 20°C) and a heat of vaporization of 14.7 cal mol^{-1} at 25 °C (Vostal, 1972). The most prevalent mineral of mercury is cinnabar (HgS). It is found in highest concentrations in volcanically active zones such as the circumpacific volcanic belt, the East Pacific Rise, and the Mid-Atlantic Ridge. The occurrence of mercury metal is rarer. In water, inorganic mercury may exist as aquo, hydroxo, halido, and bicarbonate complexes of mercuric ion, but the mercuric ion may also be adsorbed to particulate or colloidal materials in suspension (Jonasson, 1970). In soil, inorganic mercury may exist in the form of elemental mercury vapor that may be adsorbed to soil matter, at least in part. It can also exist as mercury humate complexes at pH 3–6 or as $Hg(OH)^+$ and $Hg(OH)_2$ in the pH range 7.5–8.0. The latter two species may be adsorbed to soil particles (Jonasson, 1970). Mercury in soil and water may also exist as methylmercury [$(CH_3)Hg^+$], which may be adsorbed by negatively charged particles such as clays.

Mercuric ions (Hg^{2+}) are toxic because they bind readily to exposed sulfhydryl (−SH) groups of enzyme proteins and are therefore potent nonspecific enzyme inhibitors. Their toxicity can be modulated by various organic solutes that can form mercury(II) complexes. This is of special significance when mercury toxicity for microbes is determined in growth assays (Farrell et al., 1993).

14.3 ANTHROPOGENIC MERCURY

The local mercury level in the environment may be affected by human activity. These activities include industrial operations such as the synthesis of certain chemicals like vinyl chloride and acetaldehyde that employ inorganic mercury compounds as catalysts. They also include the electrolytic production of chlorine gas and caustic soda, which employs mercury electrodes, and they also include

the manufacture of paper pulp, which makes use of phenylmercuric acetate as a slimicide (Jonasson, 1970). Some of the mercury used in these processes may accidentally pollute the environment. In agriculture, organic compounds used as fungicides to prevent fungal attack of seeds may pollute the soil. In mining, the exposure of mercury ore deposits and other deposits in which mercury is only a trace component leads to weathering and resultant solubilization, which introduces some of the mercury into the environment.

14.4 MERCURY IN THE ENVIRONMENT

As Jonasson (1970) pointed out, inorganic mercury compounds were considered less toxic in the past than organic mercury compounds, but since the discovery that inorganic mercury compounds can be converted into organic ones (e.g., methylmercury), this is no longer considered to be true. Living tissue has a high affinity for methylmercury [$(CH_3)Hg^+$]. Fish have been found to concentrate it up to 3000 times the concentration found in water. This is because methylmercury is fat- as well as water-soluble and is taken up more readily by living cells than mercuric ion. Owing to the lipid solubility of methyl mercury, nervous tissue, especially the brain, has a high affinity for it. It is also bound by inert matter, especially negatively charged particles such as clays.

Dimethylmercury [$(CH_3)_2Hg$] is volatile. It can thus enter the atmosphere from soil and water phases. The ultraviolet component of sunlight can, however, dissociate dimethylmercury into volatile elemental mercury, methane, and ethane.

Microorganisms have been shown to be intimately involved in interconversions of inorganic and organic mercury compounds (Trevors, 1986). The initial discoveries of such microbial activities were those of Jensen and Jernelöv (1969), who demonstrated the production of methylmercury from mercuric chloride ($HgCl_2$) added to lake sediment samples and incubated for several days in the laboratory. They also noted the production of dimethylmercury from decomposing fish tissue containing methylmercury or supplemented with Hg^{2+} and incubated for several weeks. Later work established that methylation was brought about by bacteria and fungi (see below). Mercury methylation is inhibited at acid pH (Steffan et al., 1988).

Microbial action on mercury compounds is a means of *detoxification*. By forming volatile elemental mercury or dimethylmercury, neither of which is water-soluble, the microbes ensure removal of mercury from their environment into the atmosphere. Even the formation of microbial methylmercury can be a form of mercury detoxification, because methylmercury can be immobilized by adsorption to negatively charged clay particles in sediment or soil, which removes it as a toxicant from the microbial environment. Similarly, the precipitation of HgS by reaction of Hg^{2+} with biogenic H_2S is a type of mercury detoxification,

because the solubility of HgS is very low ($K_{sol} = 10^{-49}$). Of all these detoxification mechanisms, formation of volatile metallic mercury has been thought to be the predominant microbial detoxification mechanism (Robinson and Tuovinen, 1984). Baldi et al. (1987) demonstrated the presence of a significant number of mercury-resistant bacteria that could reduce Hg^{2+} to Hg^0 but not methylate it at sites surrounding natural mercury deposits situated in Tuscany, Italy. Baldi et al. (1989) also isolated 37 strains of aerobic, mercury-resistant bacteria from the Fiora River in southern Tuscany, which receives drainage from a cinnabar mine. All 37 strains were able to reduce Hg^{2+} to Hg^0, but only three were also able to degrade methylmercury. None were able to generate methylmercury.

14.5 SPECIFIC MICROBIAL INTERACTIONS WITH MERCURY

Nonenzymatic Methylation of Mercury by Microbes

An early study of the biochemistry of microbial methylation of mercury involved the use of a cell extract of a methanogenic culture in the presence of low concentrations of Hg^{2+}. This extract caused the formation of $(CH_3)_2Hg$ but little methane through preferential interaction between methylcobalamin and Hg^{2+} (Wood et al., 1968). Although the production of methylcobalamin in this instance depended on enzymatic catalysis, the production of $(CH_3)_2Hg$ from the reaction of Hg^{2+} with methylcobalamin did not. This nonenzymatic nature of mercury methylation by methylcobalamin was confirmed by Bertilsson and Neujahr (1971), Imura et al. (1971), and Schrauzer et al. (1971). The nonenzymatic mechanism of mercury methylation by methylcobalamin was explained as follows (DeSimone et al., 1973):

$$Hg^{2+} \xrightarrow{CH_3B_{12}} (CH_3)Hg^{+} \xrightarrow{CH_3B_{12}} (CH_3)_2Hg \qquad (14.1)$$

According to Wood (1974), the initial methylation of Hg^{2+} in this reaction sequence proceeds 6000 times as fast as the second. However, more recent study of these reactions has indicated that the methylation of methylmercury can proceed as fast as the initial methylation of mercury (Filippelli and Baldi, 1993; Baldi, 1997). The methylation rate of methylmercury is affected by the counter ion associated with mercury. The rate is faster if sulfate is the counter ion than when it is a halogen ion such as chloride or iodide. The halogens have a greater tendency than sulfate to bind covalently to Hg^{2+}.

Dimethylmercury can also arise from a reaction of methylmercury with hydrogen sulfide (Craig and Bartlett, 1978; Baldi, 1997). In nature, hydrogen sulfide is frequently of biogenic origin, formed anaerobically by sulfate-reducing

bacteria. The transformation of methyl- to dimethylmercury can be summarized by the reactions

$$2(CH_3)Hg^+ + H_2S \rightarrow (CH_3)Hg{-}S{-}Hg(CH_3) + 2H^+ \quad (14.2a)$$

$$(CH_3)Hg{-}S{-}Hg(CH_3) \rightarrow (CH_3)_2Hg + HgS \quad (14.2b)$$

Reaction (14.2b) is slow and appears to be the rate controlling reaction (Baldi et al., 1993; Baldi, 1997).

Other studies have revealed that mercury can be nonenyzmatically methylated by microbes other than methanogens, including both aerobes and anaerobes (see review by Robinson and Tuovinen, 1984). Among anaerobes, *Clostridium cochlearium* was shown to methylate mercury contained in HgO, $HgCl_2$, $Hg(NO_3)_2$, $Hg(CN)_2$, $Hg(SCN)_2$, and $Hg(OOCCH_3)_2$ (Yamada and Tonomura, 1972a, 1972b).

Among aerobes, *Pseudomonas* spp., *Bacillus megaterium*, *Escherichia coli*, *Enterobacter aerogenes*, and others have been implicated (see summary by Robinson and Tuovinen, 1984). Even fungi such as *Aspergillus niger*, *Scopulariopsis brevicaulis*, and *Saccharomyces cerevisiae* have been found capable of mercury methylation (see Robinson and Tuovinen, 1984).

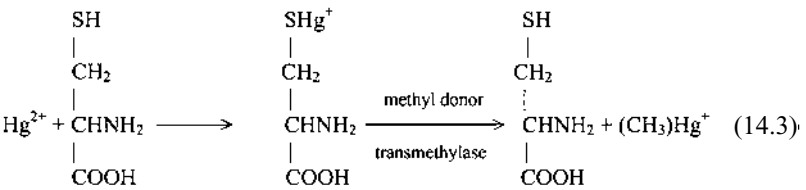
Bacteria and other microbes have been found to be able to methylate metals other than mercury. Among these metals are cadmium, lead, tin, and thallium and the metalloids selenium and tellurium (Brinckman et al., 1976; Guard et al., 1981; Schedlbauer and Heumann, 2000; Wong et al., 1975; review by Summers and Silver, 1978). Methylation of some metals may occur as a result of nonbiological transmethylation by biogenic methylated donor compounds such as trimethyltin and methyl iodide (Brinckman and Olson, 1986). Methyl halides, including methyl iodides (White, 1982; Brinckman and Olson, 1986), are produced by some marine algae and also by microorganisms associated with them (White, 1982; Manley and Dastoor, 1988) and fungi (Harper, 1985). They can readily react nonenzymatically with some metal salts (Brinckman and Olson, 1986). Trimethyltin yields carbanions that can methylate metal ions such as those of palladium, thallium, platinum, and gold, forming unstable methylated intermediates that undergo reductive demethylation to yield the metal in the elemental state. Mercuric ion reacting with trimethyltin, however, forms stable methylmercury (Brinckman and Olson, 1986).

Although in the laboratory nonenzymatic methylation appears to be favored by anaerobic conditions, partially aerobic conditions are needed in nature. This is because under in situ anaerobic conditions biogenic H_2S may prevail, and as a result, mercuric mercury will exist most probably as HgS (Fagerstrom and Jernelöv, 1971; Vostal, 1972). HgS cannot be methylated without prior conversion to a soluble Hg^{2+} salt or HgO (Yamada and Tonomura, 1972c).

Enzymatic Methylation of Mercury by Microbes

Sulfate reducers such as *Desulfovibrio desulfuricans* appear to be the principal methylators of mercury in some anoxic estuarine sediments when sulfate is limiting and fermentable organic energy sources are available (Compeau and Bartha, 1985; Benoit et al., 2001). Some other sulfate reducers also have this capacity (Benoit et al., 2001). The methyl group was shown to originate from carbon-3 of the amino acid serine. which is transferred to tetrahydrofolate (THF) to form methylene THF catalyzed by serine hydroxymethyl transferase. The methylene THF is then reduced to methyl THF by reduced ferredoxin. In the methylation of mercury, the methyl group of methyl THF is transferred to mercury via a cobalamin–protein complex, the cobalamin being the transfer agent and the protein being the catalyst (enzyme) of the methyl transfer from methyl THF to Hg^{2+} (Berman et al., 1990; Choi and Bartha, 1993; Choi et al., 1994a, 1994b). Methyl THF may also arise from formate via formyl THF, methenyl THF, and methylene THF. The normal role of the methylcobalamin–protein complex in *D. desulfuricans* is to provide the methyl group in acetate synthesis from CO_2 by the acetyl~SCoA pathway (see Chaps. 6 and 18). Hg^{2+} evidently acts as a competing methyl acceptor in acetate formation in the organism (Choi et al., 1994b). The methyl transfer from the methylcobalamin–protein complex to mercury follows Michaelis–Menten kinetics and is 600 times as fast as the uncatalyzed transfer from methylcobalamin at pH 7. These findings raise a question about the abiotic mercury methylation described in the previous section. These mercury methylation reactions should be re-examined to check whether a transmethylase is never involved in those instances.

The fungus *Neurospora crassa* uses a different mechanism of methylating mercury (Landner, 1971). In this organism, mercury forms a complex with homocysteine or cysteine nonenzymatically, and then, with the help of a methyl donor and the enzyme transmethylase, methylmercury is cleaved from this complex. The following illustrates this reaction with cysteine:



(14.3)

Suitable methyl donors are betaine or choline but not methylcobalamin.

Microbial Diphenylmercury Formation

A case of microbial conversion of phenylmercuric acetate to diphenylmercury has been reported (Matsumara et al., 1971). The reaction can be summarized as follows (where ϕ represents the phenyl moiety):

$$2\phi Hg^{+} \rightarrow \phi{-}Hg{-}\phi + \text{unknown Hg compound and a trace of } Hg^{2+} \tag{14.4}$$

Microbial Reduction of Mercuric Ion

Mercuric ion is reduced to volatile metallic mercury by a number of bacteria and fungi. Active organisms include strains of *Pseudomonas* spp., enteric bacteria, *Staphylococcus aureus*, *Thiobacillus ferrooxidans*, group B *Streptococcus*, *Streptomyces*, and *Cryptococcus* (Brunker and Bott, 1974; Komura et al., 1970; Nelson et al., 1973; Olson et al., 1981; Nakahara et al., 1985; Summer and Lewis, 1973). On the basis of mercury resistance, which correlates with Hg^{2+}-reducing ability, *Bacillus*, *Vibrio*, coryneform bacteria, *Cytophaga*, *Flavobacterium*, *Achromobacter*, *Alcaligenes*, and *Acinetobacter* (Nelson and Colwell, 1974) should be included. A significant number of *Pseudomonas* strains having Hg^{2+}-reducing ability were found unable to utilize glucose (Nelson and Colwell, 1974).

The enzyme system that catalyzes Hg^{2+} reduction to Hg^{0} involves a soluble NADPH-dependent cytoplasmic flavoprotein that is active in the presence of an excess of exogenously supplied thiols (RSH). The thiols react with Hg^{2+} to form a thiol complex (RS−Hg−RS). The thiols (in the lab they may be mercaptoethanol, dithiothreitol, glutathione, or cyteine) ensure the reduced state of mercuric reductase and the formation of Hg^{0}. The reaction catalyzed by mercuric reductase may be summarized as follows (Fox and Walsh, 1982; Foster, 1987):

$$NADPH + H^{+} + RS{-}Hg{-}SR \rightarrow NADP^{+} + Hg^{0} + 2RSH \tag{14.5}$$

In some reactions, the dimercaptyl derivative of Hg^{2+} may be replaced by a monomercaptyl or an ethylenediamine (EDTA) derivative. NADPH can be replaced by NADH with enzyme preparations from some organisms, but the preparation is then less active. The kinetics for the purified enzyme is biphasic. [See review by Robinson and Tuovinin (1984) for a more detailed discussion.] Although the reaction occurs under reducing conditions, it is performed by many obligate and facultative aerobes (e.g., Nelson et al., 1973; Spangler et al., 1973a, 1973b).

Mercuric reductase activity is not entirely substrate-specific. Besides mercuric ion, the enzyme can also reduce ionic silver and ionic gold to corresponding metal colloids (Summers and Sugarman, 1974; Summers, 1986).

Silver or gold resistance in bacteria is not, however, related to this enzyme activity (Summers, 1986).

Not all reduction of mercuric mercury observed in nature is biological (Nelson and Colwell, 1975). Chemical reduction may occur as a result of interaction with humic acid (Alberts et al., 1974).

Microbial Decomposition of Organomercurials

Phenyl- and methylmercury can be microbially converted to volatile Hg^0 by bacteria in lake and estuarine sediments and in soil (Nelson et al., 1973; Spangler et al., 1973a, 1973b; Tonomura et al., 1968). The bacteria that seem most frequently involved are mercury-resistant strains of *Pseudomonas*. Although mercury-resistant strains of other genera, such as *Escherichia coli* and *Staphylococcus aureus*, also exhibit this activity, they seem to be much less active (Nelson and Colwell, 1975). The removal of phenyl or methyl groups linked to mercuric mercury is catalyzed by a class of enzymes called mercuric lyases. They catalyze the cleavage of carbon–mercury bonds and in laboratory demonstrations require the presence of an excess of reducing agent such as L-cysteine. They release Hg^{2+}, which is then reduced to Hg^0 by mercuric reductase (Furukawa and Tonomura, 1971, 1972a, 1972b; Tezuka and Tonomura, 1976, 1978; Robinson and Tuovinen, 1984). Phenylmercuric lyase can be inducible (Nelson et al., 1973; Robinson and Tuovinen, 1984). The overall reactions can be summarized as follows:

$$\phi Hg^+ + H^+ + 2e \rightarrow Hg^0 + \phi H \tag{14.6}$$

$$(CH_3)Hg^+ + H^+ + 2e \rightarrow Hg^0 + CH_4 \tag{14.7}$$

Besides methyl- and phenylmercury compounds, some bacteria are able to decompose one or more of the following organomercurials: ethylmercuric chloride (EMC), fluorescein mercuric acetate, *para*-hydroxymercuribenzoate (pHMB), thimerosal, and merbromin (see review by Robinson and Tuovinen, 1984). The dephenylation of triphenyltin to diphenyltin by *Pseudomonas chloraphis* CNR15, *P. chloraphis* ATCC 9446, *P. fluorescens* ATCC 13525, and *P. aeruginosa* ATCC 15962 does not involve mercuric lyase but a TPT-degrading factor, which has a low molecular mass of about 1000 Da and resembles pyoverdin (Inoue et al., 2000).

Oxidation of Metallic Mercury

Elemental mercury (Hg^0) has been reported to be oxidizable to mercuric ion in the presence of certain bacteria (Holm and Cox, 1975). Whereas strains of *P. aeruginosa*, *P. fluorescens*, *E. coli*, and *Citrobacter* oxidized only small amounts of Hg^0, strains of *B. subtilis* and *B. megaterium* oxidized more significant

amounts. In none of these cases was methylmercury formed. The observed oxidation was not enzymatic but was due to reaction with metabolic products, which acted as oxidants. Even yeast extract was found to be able to oxidize Hg^0.

14.6 GENETIC CONTROL OF MERCURY TRANSFORMATIONS

In general, resistance to the toxicity of inorganic mercury compounds in bacteria is attributable to the ability to form mercuric reductase and, for certain organomercurials, mercuric lyase. However, in a strain of *Enterobacter aerogenes*, bacterial resistance to some organomercurials may be due to membrane impermeability (Pan Hou et al., 1981), and in *Clostridium cochlearium* it is due to demethylation followed by precipitation with H_2S generated by the organism (Pan Hou and Imura, 1981).

The genes encoding mercuric reductase and mercuric lyase are usually found on plasmids; i.e., they are R- or sex-factor-linked (Belliveau and Trevors, 1990; Komura and Izaki, 1971; Loutit, 1970; Novick, 1967; Richmond and John, 1964; Smith, 1967; Schottel et al., 1974; Summers and Silver, 1972; Silver and Phung, 1996; Silver, 1997). The mercury resistance determinant (gene complex) in *Bacillus cereus* RC607 was mapped by Gupta et al. (1999). Except in *Thiobacillus ferrooxidans*, the mercury resistance genes (*mer*) in all bacteria so far tested are expressed only in the presence of mercury compounds; i.e., the enzymes they code for are *inducible* (Robinson and Tuovinen, 1984). Depending on the organisms, induction of the two enzymes mercuric lyase and mercuric reductase may be *coordinated*; i.e., the two genes are under common regulatory control. In such an instance, an organomercurial induces both the lyase and the mercuric reductase (see Robinson and Tuovinen, 1984). In *T. ferrooxidans*, the mercuric resistance (Hg^r) trait appears to be constitutive (Olson et al., 1982). In a number of instances the mercury resistance trait has been found to be *transposable*; i.e., the gene complex may move to other positions on the same or other plasmids or the bacterial chromosome within a given cell (Foster, 1987). The genetic determinants in *C. cochlearium* for demethylation of methylmercury precipitation are also plasmid-encoded (Pan Hou and Imura, 1981). The demethylated mercury may be precipitated as HgS with biogenic H_2S.

Many gram-positive bacteria that are sensitive to mercury have been found to possess the gene (*merA*) that codes for the mercuric reductase enzyme. Because synthesis of mercuric reductase is inducible in these strains, their mercury sensitivity is apparently due to the lack of a determinant for a functional mercury transport mechanism into the cell (Bogdanova et al., 1992). The needed components of the mercury resistance gene complex (operon) were determined by Hamlett et al. (1992).

In nature, plasmid-determined mercury resistance in bacteria can be transferred from resistant to susceptible cells through conjugation or phage transduction among gram-negative organisms and through phage transduction among gram-positive organisms (Summers and Silver, 1972). In other words, a mercury-sensitive strain can become mercury resistant by acquiring a plasmid containing the *mer* genes from a Hg^r bacterium.

In the fungus *Neurospora crassa*, in which Hg^{2+} methylation is the basis for mercury tolerance, the mercury-resistant strain isolated by Landner (1971) appears to be a mutant that has lost control over one of the last enzymes in methionine biosythesis, so that methylation of Hg^{2+} no longer competes with methionine synthesis, as it does in the wild-type parent strain. The resistant strain could tolerate 225 mg Hg L^{-1}.

14.7 ENVIRONMENTAL SIGNIFICANCE OF MICROBIAL MERCURY TRANSFORMATIONS

The enzymatic attack of mercury compounds is not for the dissipation of excess reducing power or respiration, nor is it for the production of useful metabolites. Its function is detoxification. This is well illustrated in experiments with mercury-sensitive and -resistant strains of *Thiobacillus ferrooxidans* (Baldi and Olson, 1987). The mercury-sensitive strain could not oxidize pyrite admixed with cinnabar (HgS) in oxidation columns, whereas two resistant strains could. Yet even the resistant strains could not use cinnabar as an energy source. The mercury-resistant strains volatilized the mercury as Hg^0.

Extensive mercury methylation has been observed in mercury-polluted Clear Lake in California (Macalady et al., 2000). Analysis of the community structure in sediment samples from various locations in the lake indicated that *Desulfobacter* sp. was the dominant mercury methylator in the lower arm of the lake, where the organic carbon content was highest.

Volatile mercury (Hg^0) and dimethylmercury, owing to their high volatility and low water solubility, are readily lost to the atmosphere from the normal habitat of microbes and other creatures. Methylmercury, because of its positive charge, can become immobilized by adsorption to negatively charged clay particles in soil and sediment. The metabolic transformation to volatile forms of mercury or to methylmercury, which may become fixed in soil or sediment, protects not only the organisms actively involved in the conversion but also coinhabitants that lack this ability and are susceptible to mercury poisoning. By contrast, development of mercury resistance in microbes that is due to lessened permeability to a mercury compound benefits only those organisms that have acquired this trait.

14.8 A MERCURY CYCLE

On the basis of the interactions of microbes with mercury compounds described in the foregoing sections, it is apparent that microbes play an important role in the movement of mercury in nature, i.e., the soil, sediment, and aqueous environments. One of the main results of microbial action on mercury, whether in the form of mercuric ion or alkyl- or arylmercury ions, seems to be its volatilization as Hg^0. Methylmercury ion may also be an important end product of microbial action on mercuric ion, but it can also be an intermediate in the formation of volatile dimethylmercury. Methylmercury is more toxic to susceptible forms of life than mercuric ion, owing to the greater lipid solubility combined with its positive charge of the former. Baldi (1997) suggests that microbial activity is an important factor in homeostatic control on the level of available mercury in the environment.

A mercury cycle is outlined in Figure 14.1. Mercuric sulfide of volcanic origin slowly autoxidizes to mercuric sulfate on exposure to air and moisture and may become disseminated in soil and water through groundwater movement. Bacteria and fungi may reduce the Hg^{2+} to Hg^0, as may humic substances. The volatile mercury (Hg^0) may be adsorbed by soil, sediment, and humic substances or lost to the atmosphere. Some of the Hg^{2+} may also become methylated through the action of bacteria and fungi. Some of the positively charged mercuric ions, methylmercury ions as well as phenylmercury ions, may be fixed by negatively charged soil and sediment particles and by humic matter and thereby become immobilized. This may explain why mercury concentrations in soil are

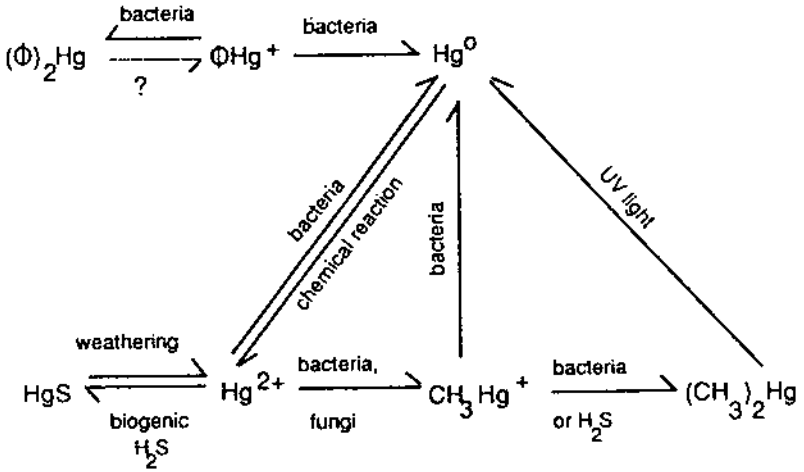


FIG. 14.1 Mercury transformations by microbes and by chemical and physical agents.

often higher in topsoil than in subsoil (Anderson, 1967). Those ions that are not fixed can be further disseminated by water movement.

Mercury methylation and demethylation have been observed to occur seasonally in the surficial sediments of the deep parts of an oligotrophic lake, with measurable amounts of the methylmercury passing into the overlying water column to be subsequently demethylated and reduced to volatile metallic mercury (Korthals and Winfrey, 1987). Methylmercury was also demethylated in the surficial sediments. During the year, methylation was most intense from mid-July through September, whereas demethylation was most active from spring to late summer (Korthals and Winfrey, 1987).

Methylation and demethylation of mercury together with mercury reduction have also been observed in estuarine sediments (Compeau and Bartha, 1984), sulfate reducers being the principal agents of methylation when the supply of sulfate is limiting (Compeau and Bartha, 1985).

Some methylmercury ions may be further methylated to volatile dimethylmercury, which readily escapes into the atmosphere from soil and water. In the atmosphere it may be decomposed into Hg^0, methane, and ethane by solar ultraviolet radiation. Phenylmercury, which usually originates from human activities, may be reduced to volatile mercury by bacteria in soil, but it may also be converted to diphenylmercury. Mercuric ion may be converted to mercuric sulfide by bacterially generated H_2S and thereby be immobilized. Whereas the formation of mercuric sulfide is strictly dependent on anaerobiosis, the methylation or reduction of mercuric mercury can occur aerobically and anaerobically.

14.9 SUMMARY

Mercuric ion (Hg^{2+}) may be methylated by bacteria and fungi to methylmercury [$(CH_3)Hg^+$]. In some bacteria this methylation is enzymatic. At least some fungi use a methylation mechanism different from that of bacteria. Methylmercury is lipid- and water-soluble and more toxic than mercuric ion. In nature, some methylmercury may, however, be adsorbed by soil or sediment, which removes it as a toxicant as long as it remains adsorbed. Some bacteria can methylate methylmercury, forming volatile dimethylmercury. This may be a form of detoxification when it occurs in soil or sediment, because this compound is water-insoluble and can escape into the atmosphere. Methylmercury as well as phenylmercury can be enzymatically reduced to volatile metallic mercury (Hg^0) by some bacteria. Phenylmercury can also be microbially converted to diphenylmercury. Most important, mercuric ion can be enzymatically reduced to metallic mercury by bacteria and fungi. This is also a form of detoxification, because the product of the reduction is volatile. Metallic mercury may also be oxidized to mercuric mercury by bacteria. However, this reaction is not enzymatic

but rather the result of interaction of metallic mercury with metabolic by-products. Microbes capable of metabolizing mercury are generally resistant to its toxic effects.

The mercury cycle in nature is under the influence of microoganisms.

REFERENCES

Alberts JJ, Schindler JE, Miller RW, Nutter DE Jr. 1974. Elementary mercury evolution mediated by humic acid. Science 184:895–897.

Anderson A. 1967. Mercury in soil. Grundförbättring 20:95–105.

Baldi F. 1997. Microbial transformation of mercury species and their importance in the biogeochemical cycle of mercury. In: Sigel A, Sigel H, eds. Metal Ions in Biological Systems. New York: Marcel Dekker, pp 213–257.

Baldi F, Olson JG. 1987. Effects of cinnabar on pyrite oxidation by *Thiobacillus ferrooxidans* and cinnabar mobilization by a mercury-resistant strain. Appl Environ Microbiol 53:772–776.

Baldi F, Olson GJ, Brinckman FE. 1987. Mercury transformations by heterotrophic bacteria isolated from cinnabar and other metal sulfide deposits in Italy. Geomicrobiol J 5:1–16.

Baldi F, Filippelli M, Olson GJ. 1989. Biotransformation of mercury by bacteria isolated from a river collecting cinnabar mine waters. Microb Ecol 17:263–274.

Baldi F, Pepi M, Filippelli M. 1993. Methylmercury resistance in *Desulfovibrio desulfuricans* strains in relation to methylmercury degradation. Appl Environ Microbiol 59:2479–2485.

Belliveau BH, Trevors JT. 1990. Mercury resistance determined by a self-transmissible plasmid in *Bacillus cereus* 5. Biol Metals 3:188–196.

Benoit JM, Gilmour CC, Mason RP. 2001. Aspects of bioavailability of mercury for methylation in pure cultures of *Desulfobulbus propionicus* (1pr3). J Bacteriol 183:51–58.

Berman M, Chase T Jr, Bartha R. 1990. Carbon flow in mercury biomethylation by *Desulfovibrio desulfuricans*. Appl Environ Microbiol 56:298–300.

Betilsson L, Neujahr HY. 1971. Methylation of mercury compounds by methylcobalamin. Biochemistry 10:2805–2808.

Bogdanova ES, Mindlin SZ, Pakrova E, Kocur M, Rouch DA. 1992. Mercuric reductase in environmental gram-positive bacteria sensitive to mercury. FEMS Microbiol Lett 97:95–100.

Brinckman FE, Olson GJ. 1986. Chemical principles underlying bioleaching of metals from ores and solid wastes, and bioaccumulation of metals from solution. In: Ehrlich HL, Holmes DE, eds. Workshop on Biotechnology for the Mining, Metal-Refining, and Fossil Fuel Processing Industries. Biotech Bioeng Symp 16. New York: Wiley, pp 35–44.

Brinckman FE, Iverson WP, Blair W. 1976. Approaches to the study of microbial transformation of metals. In: Sharpley JM, Kaplan AM, eds. Proceedings of the Third International Biodegradation Symposium. London: Appl Sci Publ, pp 916–936.

Brunker RL, Bott TL. 1974. Reduction of mercury to the elemental state by a yeast. Appl Microbiol 27:870–873.
Choi S-C, Bartha R. 1993. Cobalamin-mediated mercury methylation by *Desulfovibrio desulfuricans* LS. Appl Environ Microbiol 59:290–295.
Choi S-C, Chase T Jr, Bartha R. 1994a. Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. Appl Environ Microbiol 60:1342–1346.
Choi S-C, Chase T Jr, Bartha R. 1994b. Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. Appl Environ Microbiol 60:4072–4077.
Compeau G, Bartha R. 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. Appl Environ Microbiol 48:1203–1207.
Compeau G, Bartha R. 1985. Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediments. Appl Environ Microbiol 50:498–502.
Cotruvo JA, Vogt CD. 1990. Rationale of water quality standards and controls. In: Amer Water Works Assoc. Water Quality and Treatment. A Handbook of Community Water Supplies, 4th ed. New York: McGraw-Hill, pp 1–62.
Craig PJ, Bartlett PD. 1978. The role of hydrogen sulfide in environmental transport of mercury. Nature (Lond) 275:635–637.
DeSimone RE, Penley MW, Charbonneau L, Smith SG, Wood JKM, Hill HAO, Pratt JM, Ridsdale S, Williams JP. 1973. The kinetics and mechanism of cobalamin-dependent methyl and ethyl transfer to mercuric ion. Biochim Biophys Acta 304:851–863.
Fagerstrom T, Jernelöv A. 1971. Formation of methylmercury from pure mercuric sulfide in aerobic organic sediment. Water Res 5:121–122.
Farrell RE, Germida JJ, Huang PM. 1993. Effects of chemical speciation in growth media on the toxicity of mercury(II). Appl Environ Microbiol 59:1507–1514.
Filippelli M, Baldi F. 1993. Alkylation of ionic mercury to methylmercury and dimethylmercury by methylcobalamin: Simultaneous determination by purge-and-trap GC in line with FTIR. Appl Organometal Chem 7:487–493.
Foster TJ. 1987. The genetics and biochemistry of mercury resistance. CRC Crit Rev Microbiol 15:117–140.
Fox B, Walsh CT. 1982. Mercuric reductase: Purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulfide. J Biol Chem 257:2498–2503.
Furukawa K, Tonomura K. 1971. Enzyme system involved in decomposition of phenyl mercuric acetate by mercury-resistant *Pseudomonas*. Agric Biol Chem 35:604–610.
Furukawa K, Tonomura K. 1972a. Metallic mercury-releasing enzyme in mercury-resistant *Pseudomonas*. Agric Biol Chem 36:217–226.
Furukawa K, Tonomura K. 1972b. Induction of metallic-mercury-releasing enzyme in mercury-resistant *Pseudomonas*. Agric Biol Chem 36(Suppl 13):2441–2448.
Guard HE, Cobet AB, Coleman WM III. 1981. Methylation of trimethyltin compounds by estuarine sediments. Science 213:770–771.
Gupta A, Phung LT, Chakravarty L, Silver S. 1999. Mercury resistance in *Bacillus cereus* RC607: Transcriptional organization and two new open reading frames. J Bacteriol 181:7080–7086.
Hamlett NV, Landsdale EC, Davis BH, Summers AO. 1992. Roles of the Tn21 merT, mer P, and merC gene products in mercury resistance and mercury binding. J Bacteriol 174:6377–6385.

Harper DB. 1985. Halomethane from halide ion: A highly efficient fungal conversion of environmental significance. Nature (Lond) 315:55.

Holm HW, Cox MF. 1975. Transformation of elemental mercury by bacteria. Appl Microbiol 29:491–494.

Imura N, Sukegawa E, Pan S-K, Nagao K, Kim J-Y, Kwan T, Ukita T. 1971. Chemical methylation of inorganic mercury with methyl-cobalamin, a vitamin B_{12} analog. Science 172:1248–1249.

Inoue H, Takimura H, Fuse H, Marakami K, Kamimura K, Yamaoka Y. 2000. Degradation of triphenyltin by a fluorescent pseudomonad. Appl Environ Microbiol 66:3492–3498.

Jensen S, Jernelöv A. 1969. Biological methylation of mercury in aquatic organisms. Nature (Lond) 223:753–754.

Jonasson IP. 1970. Mercury in the natural environment: A review of recent work. Geol Surv Can, Paper 70-57, 1970.

Komura I, Izaki K. 1971. Mechanism of mercuric chloride resistance in microorganisms. I. Vaporization of a mercury resistant compound from mercuric chloride by multiple drug resistant strains of *Escherichia coli*. J Biochem (Tokyo) 70:885–893.

Komura I, Izaki K, Takahashi H. 1970. Vaporization of inorganic mercury by cell-free extracts of drug-resistant *Escherichia coli*. Agric Biol Chem 34:480–482.

Korthals ET, Winfrey MR. 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. Appl Environ Microbiol 53:2397–2404.

Landner L. 1971. Biochemical model for the biological methylation of mercury suggested from methylation studies in vivo with *Neurospora crassa*. Nature (Lond) 230:452–454.

Loutit JS. 1970. Mating systems of *Pseudomonas aeruginosa* strain I. VI. Mercury resistance associated with the sex factor (FP). Genet Res 16:179–184.

Macalady JL, Mack EE, Nelson CD, Scow KM. 2000. Sediment microbial community structure and mercury methylation in mercury-polluted Clear Lake, California. Appl Environ Microbiol 66:1479–1488.

Manley SL, Dastoor MN. 1988. Methyl iodide (CH_3I) production by kelp and associated microbes. Mar Biol (Berl) 98:477–482.

Marine Chemistry. 1971. A Report of the Marine Chemistry Panel of the Committee of Oceanography. Washington, DC: Natl Acad Sci.

Matsumara F, Gotoh Y, Brush GM. 1971. Phenyl mercuric acetate: Metabolic conversion by microorganisms. Science 173:49–51.

Nakahara H, Schottel JL, Yamada T, Miyagawa Y, Asakawa M, Harville J, Silver S. 1985. Mercuric reductase enzymes from *Streptomyces* species and group B *Streptococcus*. J Gen Microbiol 131:1053–1059.

Nelson JD Jr, Colwell RR. 1974. Metabolism of mercury compounds by bacteria in Chesapeake Bay. In: Acker RF, Brown BF, De Palma JR, eds. Proc Int Congr Mar Corros Fouling, 3rd, 1972. Evanston, IL: Northwestern Univ Press, pp 767–777.

Nelson JD, Colwell RR. 1975. The ecology of mercury-resistant bacteria in Chesapeake Bay. Microb Ecol 1:191–218.

Nelson JD, Blair W, Brinckman FE, Colwell RR, Iverson WP. 1973. Biodegradation of phenylmercuric acetate by mercury-resistant bacteria. Appl Microbiol 26:321–326.

Novick RP. 1967. Penicillinase plasmids of *Staphylococcus aureus*. Fed Proc 26:29–38.

Olson GJ, Iverson WP, Brinckman FE. 1981. Volatilization of mercury by *Thiobacillus ferrooxidans*. Curr Microbiol 5:115–118.

Olson GJ, Porter FD, Rubinstein J, Silver S. 1982. Mercuric reductase enzyme from a mercury-volatilizing strain of *Thiobacillus ferrooxidans*. J Bacteriol 151:1230–1236.

Pan Hou HSK, Imura N. 1981. Role of hydrogen sulfide in mercury resistance determined by plasmid of *Clostridium cochlearum* T-2. Arch Microbiol 129:49–52.

Pan Hou HSM, Nishimoto M, Imura N. 1981. Possible role of membrane proteins in mercury resistance of *Enterobacter aerogenes*. Arch Microbiol 130:93–95.

Richmond MH, John M. 1964. Cotransduction by a staphylococcal phage of the genes responsible for penicillinase synthesis and resistance to mercury salts. Nature (Lond) 202:1360–1361.

Robinson JB, Tuovinen OH. 1984. Mechanism of microbial resistance and detoxification of mercury and organomercury compounds: Physiological, biochemical and genetic analyses. Microbiol Rev 48:95–124.

Schedlbauer OF, Heumann KG. 2000. Biomethylation of thallium by bacteria and first determination of biogenic dimethylthallium in the ocean. Appl Organometal Chem 14:330–340.

Schottel JA, Mandel D, Clark S, Silver S, Hodges RW. 1974. Volatilization of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. Nature (Lond) 251:335–337.

Schrauzer GN, Weber JH, Beckham TM, Ho RKY. 1971. Alkyl group transfer from cobalt to mercury: Reaction of alkyl cobalamins, alkylcobaloximes, and of related compounds with mercury acetate. Tetrahedron Lett 3:275–277.

Silver S. 1997. The bacterial view of the periodic table: Specific functions for all elements. In: Banfield J, Nealson KH, eds. Interactions Between Microbes and Minerals. Rev Mineral, Vol 35. Washington, DC: Mineral Soc Am, pp 345–360.

Silver S, Phung LT. 1996. Bacterial heavy metal resistance: New surprises. Ann Rev 50:753–789.

Smith DH. 1967. R factors mediate resistance to mercury, nickel, and cobalt. Science 156:1114–1116.

Spangler WA, Spigarelli JL, Rose JM, Miller HM. 1973a. Methylmercury: Bacterial degradation in lake sediments. Science 180:192–193.

Spangler WJ, Spigarelli JL, Rose JM, Fillipin RS, Miller HM. 1973b. Degradation of methylmercury by bacteria isolated from environmental samples. Appl Microbiol 25:488–493.

Steffan RJ, Korthals ET, Winfrey MR. 1988. Effects of acidification on mercury methylation, demethylation, and volatilization in sediments from an acid-susceptible lake. Appl Environ Microbiol 54:2003–2009.

Summers AO. 1986. Genetic mechanisms of heavy-metal and antibiotic resistances. In: Carlisle D, Berry WL, Kaplan IR, Watterson JR, eds. Mineral Exploration: Biological Systems and Organic Matter. Rubey Vol 5. Englewood Cliffs, NJ: Prentice-Hall, pp 265–281.

Summers AO, Lewis E. 1973. Volatilization of mercuric chloride by mercury-resistant plasmid-bearing strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. J Bacteriol 113:1070–1072.

Summers AO, Silver S. 1972. Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J Bacteriol 112:1228–1236.

Summers AO, Silver S. 1978. Microbial transformations of metals. Annu Rev Microbiol 32:637–672.

Summers AO, Sugarman LI. 1974. Cell-free mercury(II)-reducing activity in a plasmid-bearing strain of *Escherichia coli.* J Bacteriol 119:242–249.

Tezuka T, Tonomura K. 1976. Purification and properties of an enzyme catalyzing the splitting of carbon-mercury linkages from mercury resistant *Pseudomonas* K 62-strain. J Biochem (Tokyo) 80:779–787.

Tezuka T, Tonomura K. 1978. Purification and properties of a second enzyme catalyzing the splitting of carbon-mercury linkages from mercury-resistant *Pseudomonas* K-62. J Bacteriol 135:138–143.

Tonomura K, Makagami T, Futai F, Maeda D. 1968. Studies on the action of mercury-resistant microorganisms on mercurials. I. The isolation of a mercury-resistant bacterium and the binding of mercurials to cells. J Ferment Technol 46:506–512.

Trevors JR. 1986. Mercury methylation by bacteria. J Basic Microbiol 26:499–504.

Vostal J. 1972. Transport and transformation of mercury in nature and possible routes of exposure. In: Friberg L, Vostal J, eds. Mercury in the Environment. An Epidemiological and Toxicological Appraisal. Cleveland, OH: CRC Press, pp 15–27.

White RH. 1982. Analysis of dimethyl sulfonium compounds in marine algae. J Mar Res 40:529–536.

Wong PTS, Chau YK, Luxon PL. 1975. Methylation of lead in the environment. Nature (Lond) 253:263–264.

Wood JM. 1974. Biological cycles of toxic elements in the environment. Science 183:1049–1052.

Wood JM, Kennedy FS, Rosen CG. 1968. Synthesis of methylmercury compounds by extracts of a methanogenic bacterium. Nature (Lond) 220:173–174.

Yamada M, Tonomura K. 1972a. Formation of methylmercury compounds from inorganic mercury by *Clostridium cochlearium*. J Ferment Technol 50:159–166.

Yamada M, Tonomura K. 1972b. Further study of formation of methylmercury from inorganic mercury by *Clostridium cochlearium* T-2. J Ferment Technol 50:893–900.

Yamada M, Tonomura K. 1972c. Microbial methylation of mercury in hydrogen sulfide-evolving environments. J Ferment Technol 50:901–909.

15

Geomicrobiology of Iron*

15.1 IRON DISTRIBUTION IN THE EARTH'S CRUST

Iron is the fourth most abundant element in the Earth's crust and the most abundant element in the Earth as a whole (see Chap. 2). Its average concentration in the crust has been estimated to be 5.0% (Rankama and Sahama, 1950). It is found in a number of minerals in rocks, soil, and sediments. Table 15.1 lists mineral types in which Iron is a major or minor structural component.

The primary source of iron accumulated at the Earth's surface is volcanic activity. Weathering of iron-containing rocks and minerals is often an important phase in the formation of local iron accumulations, including sedimentary ore deposits.

15.2 GEOCHEMICALLY IMPORTANT PROPERTIES

Iron is a very reactive element. Its common oxidation states are 0, +2, and +3. In a moist environment exposed to air or in aerated solution at pH values greater

* In this chapter, the old nomenclature of the Thiobacilli is used to minimize confusion when referring to the scientific literature prior to the year 2000. Based on 16S rRNA gene sequence comparison among the Thiobacilli, Kelly and Wood (2000) created the following new generic names for the acidophiles among them: *Thiobacillus thiooxidans*, *T. ferrooxidans*, and *T. caldus* have been reassigned to the genus *Acidithiobacillus*; *T. acidophilus* has been renamed *Acidiphilium acidophilum*.

TABLE 15.1 Iron-Containing Minerals

Igneous (primary) minerals	Secondary minerals	Sedimentary minerals
Pyroxenes	Montmorillonite[a] $(OH_4Si_8Al_4O_{20} \cdot nH_2O$	Siderite ($FeCO_3$)
Amphiboles		Goethite ($Fe_2O_3 \cdot H_2O$)
Olivines	Illite $(OH)_4K_y(Al_4Fe_4Mg_4Mg_6)$-$(Si_{8-y}Al_y)O_{20}$	Limonite ($Fe_2O_3 \cdot nH_2O$ or FeOOH)
Micas		Hematite (Fe_2O_3) Magnetite (Fe_3O_4) Pyrite, marcasite (FeS_2) Pyrrhotite (Fe_nS_{n+1}); $n = 5$–6 Ilmenite ($FeO \cdot TiO_2$)

[a] Montmorillonite contains iron by lattice substitution for aluminum.

than 5, its ferrous form (+2) readily autoxidizes to the ferric form (+3). In the presence of an appropriate reducing agent, i.e., under reducing conditions, ferric iron is readily reduced to the ferrous state. In dilute acid solution, metallic iron readily oxidizes to ferrous iron:

$$Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2 \tag{15.1}$$

Ferric iron may exist as a hydroxide [$Fe(OH)_3$], oxyhydroxide [FeO(OH)], or oxide [e.g., Fe_2O_3; $Fe_2O_3 \cdot H_2O$] in neutral to slightly alkaline solution but is soluble as Fe^{3+} in acid solution. Ferric hydroxide dissolves in strongly alkaline solution because of its amphoteric nature, which allows it to exist as an oxyanion [e.g., $Fe(OH)_2O^-$]. In an aqueous environment, a mixture of Fe^{3+} and metallic iron undergoes a disproportionation (dismutation) reaction resulting in the formation of ferrous iron:

$$Fe^0 + 2Fe^{3+} \rightarrow 3Fe^{2+} \tag{15.2}$$

Hydrogen sulfide reduces ferric iron readily to form ferrous iron and, when present in excess, precipitates the ferrous iron as a sulfide or disulfide:

$$2Fe^{3+} + H_2S \rightarrow 2Fe^{2+} + 2H^+ + S^0 \tag{15.3}$$

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \tag{15.4}$$

$$2Fe^{3+} + 2H_2S \rightarrow FeS_2 + Fe^{2+} + 4H^+ \tag{15.5}$$

Reactions (15.3)–(15.5) are important microbiologically (see also Chap. 19).

15.3 BIOLOGICAL IMPORTANCE OF IRON

Function of Iron in Cells

All organisms, prokaryotic and eukaryotic, single-celled and multicellular, require iron nutritionally. A small group of homolactic fermenting bacteria consisting of the lactic streptococci is the only known exception. The other organisms need iron in some enzymatic processes that involve the transfer of electrons. Examples of such processes are aerobic and anaerobic respiration in which cytochromes, special proteins that bear iron-containing heme groups, and nonheme iron-sulfur proteins play a role in the transfer of electrons to a terminal acceptor. Phototrophic organisms also need iron for ferredoxin, a nonheme iron-sulfur protein and some cytochromes that are part of the photosynthetic system. Some cells also employ iron in some of their superoxide dismutases, which convert superoxide to hydrogen peroxide by a disproportionation reaction (see Chap. 3). Most aerobic cells produce the enzymes catalase and peroxidase for catalyzing the decomposition of toxic hydrogen peroxide to water and oxygen. Prokaryotes capable of nitrogen fixation employ ferredoxin and another nonheme iron-sulfur protein (component II of nitrogenase) as well as an iron-molybdo, an iron-vanado, or an iron-iron protein (component I) of nitrogenase (see Chap. 12).

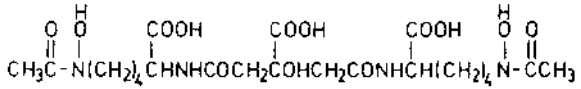
As will be discussed in Section 15.4, ferrous iron may serve as a major energy source and/or source of reducing power for certain bacteria. Ferric iron may serve as a terminal electron acceptor for a number of bacteria, usually under anaerobic conditions. As discussed in Chapter 3, ferrous iron may have served as an important reductant during the evolutionary emergence of oxygenic photosynthesis by scavenging the toxic oxygen produced in the process until the appearance of oxygen-protective superoxide dismutase. Cloud (1973) believed that the Banded Iron Formations that arose in the sedimentary record from 3.3 to 2 billion years ago are evidence for this oxygen-scavenging action by ferrous iron, but modifications of this view have been offered more recently (see discussion in Sec. 15.9). Banded Iron Formations consist of cherty magnetite (Fe_3O_4) and hematite (Fe_2O_3). From a biogeochemical viewpoint, large-scale microbial iron oxidation is important because it leads to extensive iron precipitation, and microbial reduction is important because it leads to extensive iron solubilization. In some anaerobic environments, microbial iron reduction plays an important role in the mineralization of organic carbon (iron respiration).

Iron Assimilation by Microbes

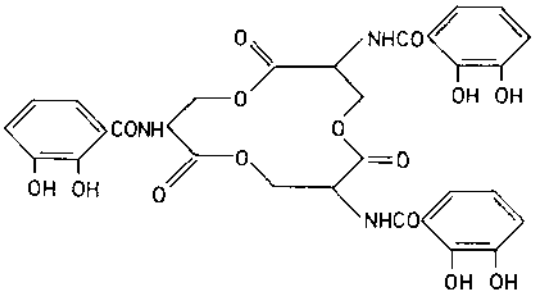
In aerobic environments of neutral pH, ferric iron precipitates readily from solution as hydroxide, oxyhydroxide, and oxide. These compounds are insoluble to various degrees at circumneutral pH, especially the latter two. This is a special problem in seawater, where iron can be a growth-limiting nutrient (e.g., Gelder,

1999; also this book, Chap. 5). As previously stated, iron is an essential trace nutrient for nearly all cellular life. Because iron can be taken into a cell only in soluble form, a number of microorganisms have acquired the ability to synthesize chelators (ligands) that help to keep ferric iron in solution at circumneutral pH or that can return it to solution in sufficient quantities from an insoluble state to meet nutritional demands. Collectively, such chelators are known as **siderophores**. They have an extremely high affinity for ferric iron but very low affinity for ferrous iron. They exhibit proton-independent binding constants for Fe^{3+} ranging from $10^{22.5}$ to 10^{49}–10^{53} (Reid et al., 1993) compared to around 10^{8} for Fe^{2+}. Examples of siderophores are enterobactin or enterochelin, a catechol derivative from *Salmonella typhimurium* (Pollack and Neilands, 1970); aerobactin, a hydroxamate derivative produced by *Enterobacter* (formerly *Aerobacter*) *aerogenes* (Gibson and Magrath, 1969); alterobactin from *Alteromonas luteoviolacea* (Reid et al., 1993); and rhodotorulic acid, a hydroxamate derivative produced by the yeast *Rhodotorula* (Neilands, 1974) (Fig. 15.1). Citrate, produced by some fungi, can also serve as a siderophore for some bacteria (Rosenberg and Young, 1974). Iron mobilization action of siderophores may be assisted by an extracellular reductant under circumneutral, aerobic conditions. Hersman et al. (2000) reported recently on mobilization of iron from hematite (Fe_2O_3) by a siderophore secreted by *Pseudomonas mendocina* and extracellular iron-reducing activity. The reducing activity was maximal under conditions of extreme iron deprivation in the absence of hematite or FeEDTA. The siderophore mobilized the iron in the form of a ferric chelate whereas the iron-reducing activity mobilized the iron in ferrous form. The authors speculated that the iron-reducing activity was due to an extracellular enzyme produced by *P. mendocina* that was similar to extracellular iron reductases produced by *Escherichia coli* and *P. aeruginosa* (Vartanian and Cowart, 1999).

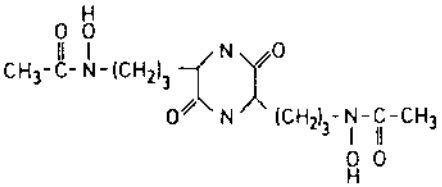
Chelated ferric iron is usually taken up by first binding to ferrisiderophore-specific receptors at the cell surface of the microbial species that produced the siderophore. In some cases, it may also bind to surface receptors of certain species of microorganisms incapable of synthesizing the desferrisiderophore (iron-free siderophore). After transport into a cell, the chelated ferric iron is usually enzymatically reduced to ferrous iron and rapidly released by the siderophore because it has only low affinity for Fe^{2+} (Brown and Ratledge, 1975; Cox, 1980; Ernst and Winkelmann, 1977; Tait, 1975). The desferrisiderophore may then be excreted again for further scavenging of iron. In some instances, the ferrisiderophore taken up by a cell is degraded first before the ferric iron is reduced to ferrous iron. The desferrisiderophore in that instance is not recyclable. In still another few instances, chelated ferric iron is exchanged with another ligand at the initial binding site at the cell surface before being transported into the cell and reduced to ferrous iron. The ferrous iron, however it originates in the cell, is immediately assimilated into heme protein or non-heme iron-sulfur protein.



(A)



(B)



(C)

FIG. 15.1 Examples of siderophores. (A) Aerobactin (bacterial); (B) enterochelin (bacterial); (C) rhodotorulic acid (fungal).

15.4 IRON AS ENERGY SOURCE FOR BACTERIA

Acidophiles

Microbes can promote iron oxidation, but this does not mean that the oxidation is always enzymatic. Because ferrous iron has a tendency to autoxidize in aerated solution at pH values above 5, it is difficult to demonstrate enzyme-catalyzed iron oxidation in near-neutral air-saturated solutions. At this time the most extensive evidence for enzyme-catalyzed iron oxidation in air-saturated solution by bacteria has been amassed at pH values below 5. The bacteria with this capacity include members in the domains Bacteria and Archaea.

Domain Bacteria: Mesophiles

A variety of acidophilic iron-oxidizing bacteria have been recognized and characterized in various acidic environments (Pronk and Johnson, 1992). They include autotrophs, mixotrophs, and heterotrophs. A more detailed discussion of some of them follows.

Thiobacillus ferrooxidans

General Traits. The most widely studied acidophilic, ferrous iron–oxidizing bacterium is *Thiobacillus ferrooxidans*. It was first isolated by Colmer et al. (1950). It is a gram-negative motile rod ($0.5 \times 1.0\,\mu m$) (Fig. 15.2). Under phosphate starvation in the laboratory, the rod-shaped cells become filamentous and exhibit some qualitative and quantitative changes in their protein (Seeger and Jerez, 1993). The organism can derive energy and reducing power from the oxidation of ferrous iron, reduced forms of sulfur (H_2S, S^0, $S_2O_3^{2-}$), metal sulfides, H_2, and formate. It gets its carbon from CO_2, and it derives its nitrogen preferentially from NH_3-N but can also use NO_3^--nitrogen (Temple and Colmer, 1951; Lundgren et al., 1964). Some strains can fix N_2 (Mackintosh, 1978; Stevens et al., 1986).

Morphologically, the cells of *T. ferrooxidans* exhibit the multilayered cell wall typical of gram-negative bacteria (Fig. 15.3) (Avakyan and Karavaiko, 1970; Remsen and Lundgren, 1966). They do not contain special internal membranes

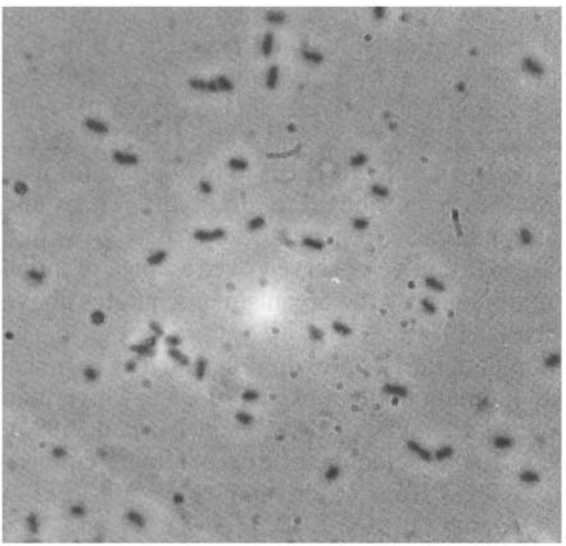


FIG. 15.2 *Thiobacillus ferrooxidans* (×5170). Cell suspension viewed by phase contrast.

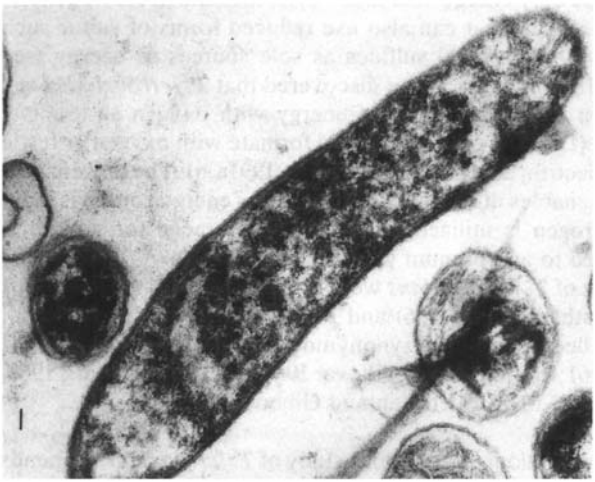


FIG. 15.3 *Thiobacillus ferrooxidans* (×30,000). Transmission electron photomicrograph of a thin section. (Courtesy of D. G. Lundgren.)

like those found in nitrifiers and methylotrophs. Cell division is mostly by constriction but occasionally also by partitioning (Karavaiko and Avakyan, 1970).

T. ferrooxidans is acidophilic. The original isolate by Temple and Colmer (1951) grew and oxidized ferrous iron in a pH range of 2.0–2.5, but these investigators did not report an overall growth range pH. The TM strain used by Silverman and Lundgren (1959a) oxidized ferrous iron optimally in a pH range of 3.0–3.6, whereas strain NCIB No. 9490, used by Landesman et al. (1966), oxidized iron optimally at pH 1.6. Razzell and Trussell (1963) gave an optimum pH for growth on ferrous iron as 2.5, and Jones and Kelly (1983) gave the pH range for growth on ferrous iron as 1–4.5. Differences in pH optima for ferrous iron oxidation and for growth on ferrous iron can be attributed to strain differences and differences in experimental conditions. In general, all strains of *T. ferrooxidans* grow and oxidize iron around pH 2. Growth can be observed over a range of initial pH of 1.5–6.0. Ferric iron produced by the organism precipitates above pH 1.9 (e.g., Buchanan and Gibbons, 1974; Starr et al., 1981).

T. ferrooxidans is mesophilic, i.e., it grows in a temperature range of 15–42°C with an optimum in the range of 30–35°C (Silverman and Lundgren, 1959a; Ahonen and Tuovinen, 1989; Niemelä et al., 1994). Ahonen and Tuovinen (1989) found that acidophilic iron-oxidizing cultures resembling *T. ferrooxidans* grew from water samples from a Finnish copper mine at incubation temperatures of 4, 7, 10, 13, 16, 19, 28, and 37°C but not at 46°C. Optimum rates of iron oxidation by resting cells of *T. ferrooxidans* have been observed around 40°C

with at least some strains (Silverman and Lundgren, 1959b; Tuttle and Dugan, 1976). A linear increase in ferrous iron oxidation rates was detected between 4 and 28°C with the Finnish cultures (Ahonen and Tuovinen, 1989).

As already noted, iron is not the only energy source for *T. ferrooxidans*. As its generic name *Thiobacillus* implies, it can also use reduced forms of sulfur such as H_2S, S^0, $S_2O_3^{2-}$, and some metal sulfides as sole sources of energy (see Chaps. 18 and 19). More recently it was discovered that *T. ferrooxidans* can also use H_2 as a sole source of energy with oxygen as terminal electron acceptor (Drobner et al., 1990) and formate with oxygen or ferric iron as terminal electron acceptor (Pronk et al., 1991a, 1991b). The key enzyme hydrogenase that enables utilization of H_2 as energy source is inducible. The hydrogenase from *T. ferrooxidans* strain 19859 has been purified and recovered as 100 and 200 kDa units containing 6.02 mol Fe and 0.72 mol Ni per mole of enzyme. The enzyme was made up of two subunits of 64 and 34 kDA molecular mass, respectively, occurring in a 1 : 1 ratio in the enzyme (Fischer et al., 1996). When H_2 is utilized, the optimal pH range for growth was 3.0–5.8 as opposed to an optimum of pH 2.0 on ferrous iron.

Some strains of *T. ferrooxidans* were originally named *Ferrobacillus ferrooxidans* (Leathen et al., 1956) and *F. sulfooxidans* (Kinsel, 1960). These have since been considered synonymous with *T. ferrooxidans* (Unz and Lundgren, 1961; Ivanov and Lyalikova, 1962; Hutchinson et al., 1966; Kelly and Tuovinen, 1972; Buchanan and Gibbons, 1974).

Laboratory Cultivation. Laboratory study of *T. ferrooxidans* depends on the ease of culturing. Table 15.2 lists the ingredients of four liquid media, of which the 9 K and T&K media are among the ones that have been widely used. For purification, cloning, and enumerating viable cells in a culture, gelled (solid) medium is essential. Use of standard agar or silica gel has not been successful. Tuovinen and Kelly (1973) suggested that galactose from the agar is inhibitory to the organism. Modified 9 K medium gelled with agarose or purified agar will, however, support growth of most strains. On such media, the organism forms rust-colored colonies with varying morphology, depending on the strain. The colonies usually develop in 1–2 weeks (Manning, 1975; Mishra and Roy, 1979; Mishra et al., 1983; Holmes et al., 1983). Another approach, which has not been extensively used, is to grow *T. ferrooxidans* cells on membrane filters (Sartorius or Millipore types) on T&K medium solidified with 0.4% Japanese agar at an initial pH of 1.55 and incubating at 20°C (Tuovinen and Kelly, 1973). Rust-colored colonies of 1.0–1.5 mm diameter develop after 2 weeks.

Johnson et al. (1987) used the following gelled medium for concurrent isolation and enumeration of *T. ferrooxidans* and acidophilic heterotrophic bacteria that usually accompany *T. ferrooxidans* in nature. The medium contains 1 part by volume of 20% ferrous sulfate solution adjusted to pH 2.0 or 2.5, 14

TABLE 15.2 Media for Cultivating *T. ferrooxidans*

Ingredient	Quantity of ingredients ($g\,L^{-1}$)			
	9 K[a]	T&K[b]	L[c]	T and C[d]
$(NH_4)SO_4$	3.0	0.4	0.15	0.5
KCl	0.1	—	—	—
K_2HPO	0.5	0.4	0.05	—
$MgSO_4 \cdot 7H_2O$	0.5	0.4	0.5	1.0
Ca $(NO_3)_2$	0.01	—	0.01	—
$FeSO_4 \cdot 7H_2O$	44.22	33.3	1.0	129.1

[a] 9 K medium of Silverman and Lundgren (1959a). $FeSO_4$ is dissolved in 300 mL of distilled water and filter-sterilized. The remaining salts are dissolved in 700 mL of distilled water and autoclaved after adjustment of the pH with 1 mL of 10 N H_2SO_4.
[b] Medium of Tuovinen and Kelly (1973). The salts are dissolved in 0.11 N H_2SO_4. The medium is sterilized by filtration.
[c] Medium of Leathen et al. (1956).
[d] Medium of Temple and Colmer (1951). The pH of this medium is adjusted to 2.0–2.5 with H_2SO_4. The medium is sterilized by filtration.

parts by volume of a basal salts–tryptone soy broth solution consisting of (in $g\,L^{-1}$) $(NH_4)_2SO_4$, 1.8; $MgSO_4 \cdot 7H_2O$, 0.7; tryptone soy broth (TSB, Oxoid Ltd., Basingstoke, U.K.), 0.35, the solution adjusted to pH 2.0, 2.5, or 3.0; and 5 parts by volume of 0.7% agarose type 1 (Sigma Ltd, St. Louis, MO). The best final pH values for optimal recovery of *T. ferrooxidans* were 2.7 and 2.3. A modification of this gelled medium that also supported the growth of moderately thermophilic, acidophilic iron-oxidizing bacteria was developed by Johnson and McGinness (1991). In this medium the final concentration of iron was lowered from 1% (33 mM) to 25 mM, and the concentration of argarose type I was lowered from 0.7% to 0.5%. Addition of some trace elements to the medium was found beneficial for some organisms. For culturing, the final medium was dispensed in two layers in Petri dishes. The bottom layer (~20 mL) consisted of medium that had been preinoculated with a desired acidophilic heterotroph, and a thin top layer (~10 mL) consisted of sterile medium. The purpose of the bacteria in the bottom layer was to have them consume as much as possible of any sugars that are contributed by the argarose and that might suppress the growth of iron oxidizers.

Facultative Heterotrophy. A number of claims have appeared in the literature that some strains of *T. ferrooxidans* can be adapted to grow heterotrophically if glucose is used instead of ferrous iron as the sole source of energy and carbon (Lundgren et al., 1964; Shafia and Wilkinson, 1969; Shafia et al., 1972; Tabita and Lundgren, 1971a; Tuovinen and Nicholas, 1977; Sugio et al.,

1981). Some of these strains could be reverted to ferrous iron–dependent autotrophy, whereas others could not. The discovery that some cultures of *T. ferrooxidans* contained heterotrophic satellite organisms (Zavarzin, 1972; Guay and Silver, 1975; Mackintosh, 1978; Arkesteyn, 1979; Harrison, 1981, 1984; Johnson and Kelso, 1983; Lobos et al., 1986; Wichlacz et al., 1986) cast serious doubt on the existence of an ability of some *T. ferrooxidans* strains to grow heterotrophically.

Facultative Mixotrophy. At least some strains of *T. ferrooxidans* seem to be capable of facultative mixotrophy. Barros et al. (1984) found that ferrous iron oxidation by strain FD1 was faster in the absence of glucose than in its presence and was increased as the ratio of iron to glucose was increased. At an initial Iron/glucose concentration ratio of 5 : 9, neither iron nor glucose was utilized. Even 0.5 g of glucose per liter in a medium with 9.0 g of iron per liter inhibited iron oxidation, but lowering the initial iron concentration from 9 g L^{-1} resulted in simultaneous use of Fe and glucose. At an initial concentration ratio of 7 : 5, a slight lag occurred during which only iron was oxidized, but when the initial concentrations of ferrous iron and glucose were 5 g L^{-1} each, iron and glucose were utilized concurrently by strain FD1 from the start. The authors showed that on the basis of mole percent GC ratios, the cultures of FD1 grown autotrophically on iron and mixotrophically on iron plus glucose were identical.

Consortia with T. ferrooxidans. The existence of satellite organisms that appear to live in close association with *T. ferrooxidans* was first reported by Zavarzin (1972). As noted above, confirmation of their existence soon followed. Indeed, taxonomically different organisms were isolated from different *T. ferrooxidans* consortia. Zavarzin (1972) isolated his organisms in modified Leathen's medium in which yeast extract (0.01–0.02%) replaced $(NH_4)_2SO_4$ and the pH was adjusted to 3–4. The organism was morphologically distinct from *T. ferrooxidans*, being a single rod. It could not oxidize iron even though it required its presence at high concentration in the medium. It was acidophilic (optimum pH range 2–3). It required yeast extract but did not grow in an excess of it. Glucose, sucrose, fructose, ribose, maltose, xylose, mannitol, ethanol, and citric, succinic, and fumaric acids could serve as energy sources. The organism was originally isolated from peat in an acid bog. It resembled *Acetobacter xylinum* but grew at lower pH than the latter and exhibited only a weak ability to form acetic acid and ethanol.

Guay and Silver (1975) derived a satellite organism from a culture of *T. ferrooxidans* strain TM by successive subculturing in 9 K medium containing increasing glucose concentrations from 0.1% to 1.0% and concomitant decreases in Fe^{2+} from 9000 ppm to 10 ppm in four steps. They isolated a pure culture of the satellite organism, which they named *Thiobacillus acidophilus*, using 9 K basal salts–glucose agar that contained 1 ppm ferrous iron at pH 4.5 and

incubating it at 25°C. The pure culture consisted of a gram-negative motile rod (0.5–0.8 μm × 1.0–1.5 μm) and was morphologically not very distinct from *T. ferrooxidans*. Physiological study of *T. acidophilus* by Guay and Silver (1975) and subsequently by Norris et al. (1986), Mason et al. (1987), and Mason and Kelly (1988) showed the organism to be capable of heterotrophic growth in suitable medium and also capable of mixotrophic growth on tetrathionate plus glucose. In addition, it was found to grow autotrophically in media with elemental sulfur (S^0), thiosulfate, trithionate, or tetrathionate as energy source and CO_2 as carbon source. The organism is incapable of oxidizing Fe^{2+} or metal sulfides. It is able to use D-ribose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, D-mannitol, sucrose, citrate, malate, *dl*-aspartate, and *dl*-glutamate as carbon and energy sources in 9 K basal medium without Fe^{2+}. It cannot use D-mannose, L-sorbose, L-rhamnose, ascorbic acid, lactose, D-maltose, cellobiose, trehalose, D-melobiose, raffinose, acetate, lactate, pyruvate, glyoxalate, fumarate, succinate, mandelate, cinnamate, phenylacetate, salicylate, phenol, benzoate, phenylalanine, tryptophane, tyrosine, or proline.

Like *T. ferrooxidans*, *T. acidophilus* is acidophilic (pH range 1.5–6.0, optimum 3.0). Its DNA has a GC ratio of 62.9–63.2 mol%, which is distinctly different from that of *T. ferrooxidans*, whose GC ratio is 56.1 mol%. Differences in key enzymes such as glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, fructose 1,6-diphosphate aldolase, isocitric dehydrogenase, 2-ketoglutarate dehydrogenase, NADH : acceptor oxidoreductase, thiosulfate-oxidizing enzyme, and rhodanese between *T. ferrooxidans* and *T. acidophilus* were also noted. The ribulose 1,5-bisphosphate carboxylase level for CO_2 fixation in *T. acidophilus* depends on whether the organism is growing mixotrophically or autotrophically. Glucose in mixotrophic cultures can partially repress the enzyme (Mason and Kelly, 1988). What is puzzling about *T. acidophilus* is that it was carried for years as a satellite of *T. ferrooxidans* TM in 9 K iron medium without an organic supplement. The explanation may be as given by Arkesteyn and DeBont (1980), that *T. acidophilus* is oligotrophic. In coculture with *T. ferrooxidans*, it lives at the expense of organic excretions (organic acids, alcohols, amino acids) from *T. ferrooxidans* that inhibit the growth of *T. ferrooxidans* if they build up in the growth medium.

Other satellite organisms that have been found associated with some *T. ferrooxidans* cultures include *Acidiphilium cryptum* (Harrison, 1981), *A. organovorum* (Lobos et al., 1986), *A. angustum*, *A. facilis*, and *A. rubrum* (Wichlacz et al., 1986). *Acidiphilium cryptum* is a heterotrophic, motile, gram-negative rod (0.3–0.4 μm × 0.5–0.8 μm) that grows in very dilute organic media in a pH range of 1.9–5.9. The organism is an oligotroph whose growth is inhibited at high organic nutrient concentrations. Its GC content is 68–70 mol%, which clearly distinguishes it from *T. ferrooxidans* and *T. acidophilus*. It cannot use reduced forms of sulfur or ferrous iron as an energy source and is inhibited by as little as

0.01% acetate. *A. organovorum* differs from *A. cryptum* by its lower GC ratio (64 mol%) and especially by its faculative oligotrophy. The *Acidiphilium* species isolated by Wichlacz et al. (1986) differ from *A. cryptum* in cell size, pigment production, nutritional traits, and genome homology. In support of the theory of Arkesteyn and DeBont (1980) concerning the role of *T. acidophilus* in nature in relation to its association with *T. ferrooxidans*, Harrison (1984) showed that growth of *T. ferrooxidans* is enhanced in the presence of *A. cryptum* when 0.004% pyruvate is present in the growth medium. Pyruvate can be used by *A. cryptum* as carbon and energy source but inhibits growth of *T. ferrooxidans* at a critical concentration even though *T. ferrooxidans* excretes pyruvate during its growth (Schnaitman and Lundgren, 1965).

Genetics. Understanding of the genetics of *T. ferrooxidans* lagged behind that of many other bacteria. One reason for this was the past difficulty of culturing the organism on solid media to obtain pure cultures to select different wild-type and mutant strains. Another reason was that the acidophilic nature of the organism made the application of typical molecular biological techniques difficult.

With the development of new culture techniques, significant advances in unraveling the genetics of *T. ferrooxidans* have been made in the last 15 years or so. For instance, in the older literature, reports appeared of "training" *T. ferrooxidans* to tolerate high concentrations of toxic metal ions such as those of Cu, Ni, Co, and Pb by serial subculture in the presence of increasing amounts of the toxicants (e.g., Marchlewitz et al., 1961; Tuovinen et al., 1971; Kamalov, 1972; Tuovinen and Kelly, 1974). Many interpreted this phenomenon on the basis of spontaneous mutation and selection. However, considering the relatively rapid rate at which these cultures developed tolerance for these metals and their limited growth yields in appropriate media, this interpretation was questioned by some. Pure cultures of rapidly growing heterotrophs acquire resistance to a toxicant more gradually by a spontaneous mutation and selection process. The recent discovery of mobile genetic elements in the genome of many *T. ferrooxidans* strains offers a more plausible explanation for their rapid acquisition of metal resistance and for phenotypic switching with respect to iron oxidation (Holmes et al., 1989; Yates and Holmes, 1987; Schrader and Holmes, 1988; Holmes et al., 2001).

Like most bacteria, *T. ferrooxidans* contains most of its genetic information on a *chromosome*, and like many bacteria, most strains of *T. ferrooxidans* also contain some nonessential genetic information on extrachromosomal DNA called **plasmids** (Mao et al., 1980; Martin et al., 1981; Rawlings et al., 1986). Some of the chromosomal and plasmid genes have been mapped and characterized (Rawlings and Kusano, 1994). These include genes of nitrogen metabolism, such as glutamine synthetase (see Rawlings and Kusano, 1994) and nitrogenase

(e.g., Pretorius et al., 1986, 1987; Rawlings and Kusano, 1994), genes involved in CO_2 fixation and in energy conservation (see Rawlings and Kusano, 1994), genes responsible for resistance to mercury, and other genes (see Rawlings and Kusano, 1994). The genomes of different strains of *T. ferrooxidans* and *T. thiooxidans* have been compared (Harrison, 1982, 1986). The 13 strains of *T. ferrooxidans* that were examined fell into different DNA homology groups. Though genetically related, they were not identical. None showed significant DNA homology with six strains of *T. thiooxidans*, indicating little genetic relationship. The *T. thiooxidans* strains fell into two different DNA homology groups. On the basis of 16 S rRNA relationships, *T. ferrooxidans* belongs to the Proteobacteria, most to the β-subgroup, but at least one strain to the γ-subgroup (see Rawlings and Kusano, 1994). A gapped genome sequence has now been determined for *Thiobacillus ferrooxidans* strain ATCC 23270 (Selkov et al., 2000).

After many failures, it finally became possible under laboratory conditions to introduce genes into strains of *T. ferrooxidans* that lacked them, using bacterial transformation via electroporation and bacterial conjugation, and having them expressed by the recipient strains. Thus, the mercury resistance gene complex (*mer* operon) was introduced into a mercury-sensitive strain of *T. ferrooxidans* (Kusano et al., 1992). Heterogeneous arsenic resistance genes on plasmids were transferred from a strain of *Escherichia coli* (heterotroph) to an arsenic-sensitive strain of *T. ferrooxidans* by filter-mating (Peng et al., 1994). To what extent gene transfer occurs among *T. ferrooxidans* stains in nature is still unknown.

The Energetics of Ferrous Iron Oxidation. Oxidation of ferrous iron does not furnish much energy on a molar basis, compared, for instance, to oxidation of glucose. In the past, estimates of free energy change (ΔG°) for iron oxidation ranged around 10 kcal mol^{-1} (41.8 kJ mol^{-1}), as calculated, for example, by Baas Becking and Parks (1927) from the equation

$$4FeCO_3 + O_2 + 6H_2O \rightarrow 4Fe(OH)_3 + 4CO_2$$
$$(\Delta G^\circ_{r,298} = -40 \text{ kcal or } -167.2 \text{ kJ}) \qquad (15.6)$$

Another examination of the question of free energy yield from ferrous iron oxidation by Lees et al. (1969), who assumed that the reaction was:

$$Fe^{2+} + H^+ + 0.25O_2 \rightarrow Fe^{3+} + 0.5H_2O \qquad (15.7)$$

and who took into account the effects of pH and ferric iron solubility on the reaction, led to the following equation for estimating ΔG°_r between pH 1.5 and 3:

$$\Delta G^\circ_r = -1.3(7.7 - \text{pH} - 0.17) \qquad (15.8)$$

From this equation it may be calculated that the ΔG°_r at pH 2.5 is -6.5 kcal mol^{-1} or -27.2 kJ mol^{-1}, barely enough for the sysnthesis of 1 mol of ATP (which requires about 7 kcal mol^{-1} or 29.3 kJ mol^{-1}). If we assume that it takes 120 kcal

TABLE 15.3 Estimates of Free Energy Efficiency of Carbon Assimilation by Iron-Oxidating Thiobacilli

Efficiency (%)	Age of cells	Reference
3.2	17 days	Temple and Colmer (1951)
30	?	Lyalikova (1958)
4.8–10.6	?	Beck and Elsden (1958)
20.5 ± 4.3	Late log phase	Silverman and Lundgren (1959b)

of energy to assimilate 1 mol of carbon at 100% efficiency (Silverman and Lundgren, 1959b), then approximately 18.5 mol of Fe would have to be oxidized to incorporate this much carbon. However, *T. ferrooxidans* is not 100% efficient in using energy available from iron oxidation. An early experimental estimate of the true efficiency of carbon assimilation at the expense of Fe^{2+} oxidation was 3.2% (Table 15.3) (Temple and Colmer, 1951). At that efficiency level, it would take 577 mol of ferrous iron. Taking the efficiency determination of Silverman and Lundgren (1959b) given in Table 15.3, a consumption of 90.1 mol of ferrous iron to assimilate 1 mol of carbon would be predicted. This is greater than the consumption of 50 mol observed by Silverman and Lundgren (1959b) and raises the question as to whether the assumption that it takes 120 kcal in these cells to assimilate 1 mol of carbon is correct. However, the estimate of 90.1 mol of Fe^{2+} per mole of carbon approaches the results of another experiment (Beck, 1960), in which about 100 mol of Fe^{2+} had to be oxidized to fix 1 mol of CO_2 by a strain of *T. ferrooxidans*. No matter what the actual efficiency of energy utilization, the observed ratios of moles of Fe^{2+} oxidized to moles of CO_2 assimilated illustrate that large amounts of ferrous iron have to be oxidized to satisfy the energy requirements for growth of these organisms.

Iron-Oxidizing Enzyme System in T. ferrooxidans. Significant advances have been made in elucidating the enzymatic mechanism of iron oxidation in *T. ferrooxidans*. Kinetic studies with whole cells of the organism yielded apparent K_m values of 2.2 mM and for cell-free preparations, 5.6 mM (pH optimum 3.5) (Ingledew, 1982).*

Ferric iron resulting from Fe^{2+} oxidation causes product inhibition and limits growth of *T. ferrooxidans* as it builds up in the medium (Kelly and Jones, 1978; Jones and Kelly, 1983; Kovalenko et al., 1982). The inhibitory effect can be modulated by a change in temperature. Increasing temperature decreases the

* K_m is defined by the Michaelis–Menten equation $v = V_{max}[S]/([S] + K_m)$, where v is the reaction velocity, V_{max} the maximal velocity, [S] the initial substrate concentration, and K_m a constant (Segel, 1975).

inhibitory effect. Physiological age also has an effect on iron susceptibility, lag phase cells being more sensitive to inhibition than log (exponential) phase cells (Kovalenko et al., 1982). Ferrous iron may cause substrate inhibition in chemostat growth (Jones and Kelly, 1983).

A requirement for sulfate ions by the iron oxidizing system of *T. ferrooxidans* was established by Lazaroff (1963). Changes in SO_4^{2-} concentration as well as pH affect the values of V_{max} but not K_m. Even when cells were adapted to the presence of Cl^-, Cl^- could not fully replace SO_4^{2-} (Lazaroff, 1963; see also Kamalov, 1967; Vorreiter and Madgwick, 1982). In at least one instance, SO_4^{2-} could be partially replaced by HPO_4^{2-} or $HAsO_4^-$ but not by BO_3^-, MoO_4^{2-}, NO_3^-, or Cl^- ions. Formate and MoO_4^{2-} ions inhibited iron oxidation (Schnaitman et al., 1969). Selenate could replace sulfate in iron oxidation by *T. ferrooxidans* but did not permit its growth. In the presence of sulfate or selenate, iron oxidation was enhanced by tellurate, tungstate, arsenate, or phosphate (Lazaroff, 1977). A role of sulfate in iron oxidation by *T. ferrooxidans* appears to be the stabilization of the hexa-aquated complex of Fe^{2+}, which serves as substrate for its iron-oxidizing enzyme system. Selenate can replace sulfate completely as anionic stabilizer, and tellurate, tungstate, arsenate, and phosphate can replace it partially (Lazaroff, 1983). The extensive formation of jarosite, a crystalline basic iron sulfate, in the presence but not in the absence of *T. ferrooxidans* suggests that in nonbiological oxidation of ferrous iron, water displaces sulfate in the ferric product (Lazaroff et al., 1982, 1985).

One currently accepted model for the iron-oxidizing system in *T. ferrooxidans* is illustrated in Figure 15.4A. According to this model, bulk-phase Fe^{2+} is oxidized at the outer surface of the outer membrane of the cell envelope by transfer of an electron to a structurally bound iron in the outer membrane that is in the +3 oxidation state and is reduced to the +2 state by the transfer. The structurally bound iron has been called polynuclear iron (Ingledew, 1986). The resultant bound ferrous iron in the outer membrane gives up its electron to the copper protein rusticyanin catalyzed by an as yet unidentified enzyme (X). The rusticyanin in turn transfers its acquired electrons to periplasmic c-type cytochrome. The reduced c-type cytochrome then binds to the outer surface of the plasma membrane, allowing for the transfer of the electrons across the membrane to cytochrome oxidase (cytochrome a_1) located on the inside surface of the plasma membrane. The reduced cytochrome oxidase then reacts with O_2, leading to the formation of water. The inclusion of the catalytic component (X) in the model in Figure 15.4A is necessary because the kinetics for electron transfer from Fe(II) to periplasmic cytochrome c are otherwise too slow to explain the observed rate of iron oxidation by intact cells (Ingledew, 1986; Cox and Boxer, 1986; Blake and Shute, 1987). Fry et al. (1986) claimed that they found evidence for such an enzyme in the form of a non-heme iron protein. The suggestion by the model that outer membrane–bound iron may be the initial electron acceptor is

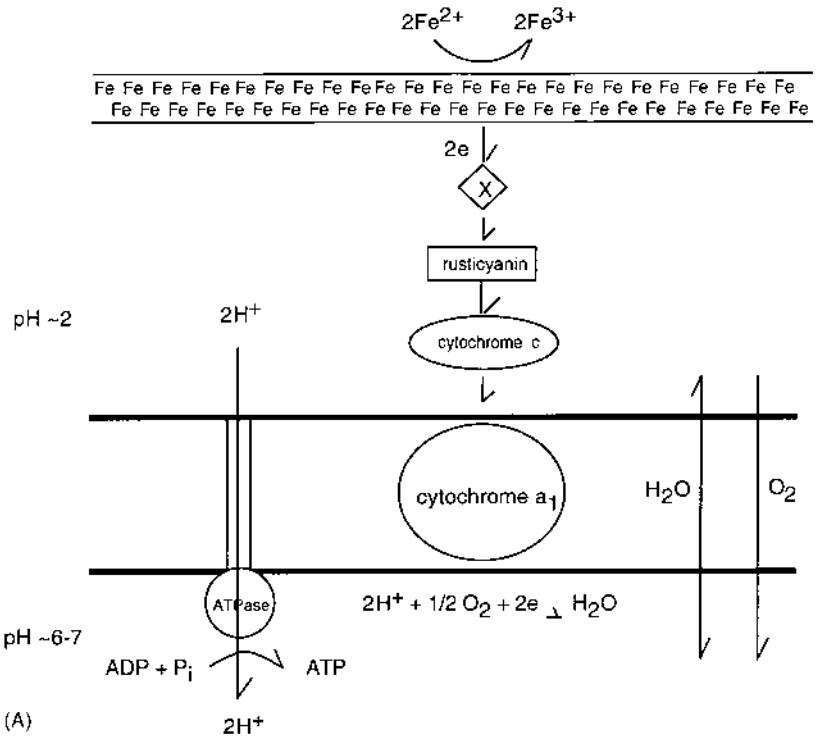


FIG. 15.4 Alternative models of bioenergetic mechanisms of iron oxidation in *Thiobacillus ferrooxidans*. (A) Model as proposed by Ingledew et al. (1977); for discussion see text. (B) One version of a model as proposed by Blake et al. (1992); for discussion see text. The periplasmic cytochrome c shown in (A) appears as a component of the outer membrane in this model. It would function in place of the polynuclear iron in (A) in transferring electrons from Fe^{2+} or other electron donors in the bulk phase to a rusticyanin in the periplasm. In an alternative model proposed by Blake et al. (1992) (not diagrammed here), the cytochrome c serves as an electron shuttle between the outer membrane and rusticyanin in the periplasm. The reaction sequences are based on kinetic studies by Blake et al. (1992).

based on a proposal by Dugan and Lundgren (1965) that some iron is bound in the cell envelope of *T. ferrooxidans*. It is also based on the finding by Agate and Vishniac (1970) that phosphatidyl serine in the membrane may be the site of this binding.

Alternative models for the iron-oxidizing system in *T. ferrooxidans* have been proposed. The most plausible of the alternative models is one based on direct observations by Blake et al. (1992) of rapid electron transfer from a molar

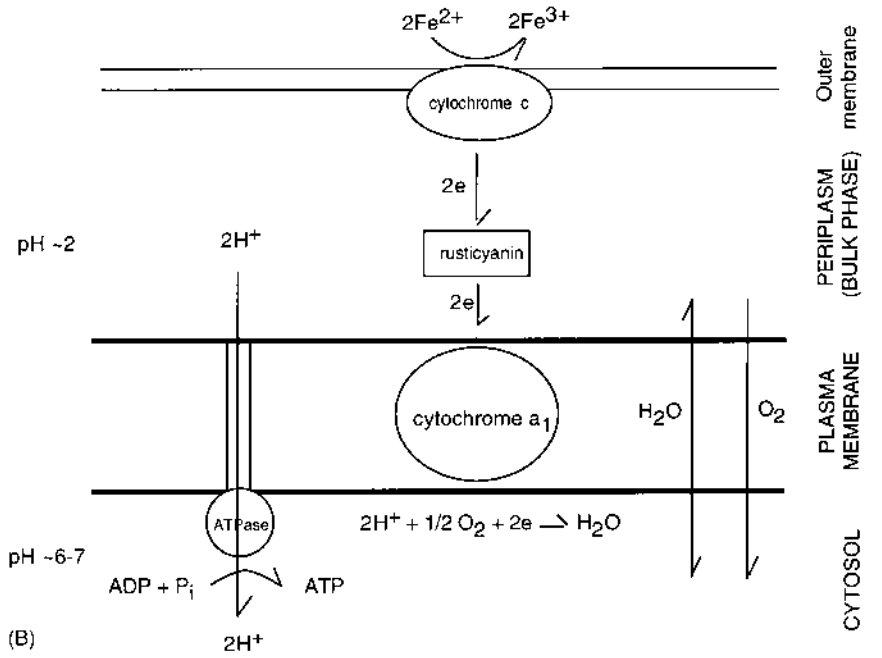


FIG. 15.4 (*continued*)

excess of Fe^{2+} to rusticyanin catalyzed by a partially purified iron-rusticyanin oxidoreductase that appeared to consist of some form of cytochrome c (Fig. 15.4B). The reaction depended on the presence of sulfate ions. The kinetics of iron oxidation was consistent with that observed with intact cells. Polynuclear iron in the outer membrane and the postulated enzyme X are not required in this model. But the absence of such involvement requires an alternative mechanism for transporting electrons from bulk-phase Fe^{2+} into the periplasm. If the iron-rusticyanin oxidoreductase were located in the outer membrane of *T. ferrooxidans*, it could serve this function. Such a location would resemble that of MnO_2 reductase and of ferric reductase in anaerobically grown *Shewanella putrefaciens* and in *Geobacter sulfurreducens*, which convey electrons from an intracellular electron donor to extracellur Fe(III) oxide or Mn(IV) oxide in contact with the outer membrane (see discussion in Chap. 16). Evidence for a satisfactory rate of electron transfer from rusticyanin to cytochrome a_1 ($38\,sec^{-1}$) was previously presented by Yamanaka et al. (1991), cited by Blake et al. (1992). Whether the model of Ingledew et al. (1977) or that of Blake et al. (1992) is most representative of the actual process of iron oxidation in intact cells of *T. ferrooxidans* remains to be determined (Blake et al., 1993).

Evidence for the involvement of the cytochrome system in the oxidation of iron by *T. ferrooxidans* was first presented by Vernon et al. (1960) and subsequently in a more detailed analysis by Tikhonova et al. (1967). The basis for the currently most plausible models of iron oxidation by *T. ferrooxidans* emerged from the discovery of the copper protein rusticyanin in its periplasm (Cobley and Haddock, 1975; Cox and Boxer, 1978, 1986; Ingledew et al., 1977; Ingledew and Houston, 1986). Blaylock and Nason (1963) separated an iron-cytochrome c reductase from a particulate iron oxidase preparation from *Ferrobacillus* (now *Thiobacillus*) *ferrooxidans*. Yates and Nason (1966a, 1966b) believed the reductase to be a DNA-containing enzyme protein, but Din et al. (1967a) showed it to be an RNA-containing enzyme. The location of this enzyme in the intact cell remains unclear. If its location is in the periplasm of *T. ferrooxidans* and it can react with rusticyanin, it may be the missing enzyme that catalyzes the transfer of electrons from the outer membrane–bound iron to rusticyanin in the model of Ingledew et al. (1977). On the other hand, Fukumori et al. (1988) isolated an Fe(II)-oxidizing enzyme from a *T. ferrooxidans* strain that conveyed electrons from Fe(II) to cytochrome c-552 but not to rusticyanin. Whether it is related to Din's iron-cytochrome c reductase is not known. Thus, major questions remain to be answered about Fe(II) oxidation by *T. ferrooxidans*.

Mansch and Sand (1992) described a membrane-bound iron-oxidizing system in *T. ferrooxidans* strain F 427 that include cytochromes of the a_1, b, and c type. The c-type cytochrome consisted of at least three different acid-stable forms with M_r values of 60,000, 30,000, and 25,000, respectively, an acid-stable protein with noncovalently bound heme with an M_r value of 20,000, and an acid-

stable protein with an M_r value of 18,000, which probably was rusticyanin. Sulfur-grown cells of this strain contained aa_3-type cytochrome in addition to the others, but cytochromes b and aa_3 were acid-labile. The investigators proposed that ferrous iron oxidation in *T. ferrooxidans* involves more than one key enzyme.

Energy Coupling in Iron Oxidation by T. ferrooxidans. Energy coupling in iron oxidation is best understood in terms of a chemiosmotic mechanism (Ingledew, 1982; Ingledew et al., 1977). Such a mechanism implies that a proton motive force is set up across the plasma membrane owing to charge separation across the two sides of the membrane. The proton motive force results from a pH gradient generated from the higher proton concentration in the acid periplasm relative to the near-neutral cytoplasm of an active *T. ferrooxidans* cell and from a transmembrane electric potential. The transfer of electrons to O_2 via the electron transport system results in pumping of protons from the cytoplasm into the periplasm, which together with the protons formed in the hydrolysis of ferric iron generated in the oxidation is a cause of the proton gradient. Energy coupling, i.e., ATP synthesis, results from the fact that the plasma membrane is impermeable to protons in the periplasm except at the sites where adonosine 5′-triphosphatase (ATPase) is anchored in the membrane. ATPase contains a proton channel that allows passage of protons in the directions of the cytoplasm. As a result of this proton movement, the ATPase causes the synthesis of ATP through the reaction

$$\mathrm{ADP} + \mathrm{P_i} \rightarrow \mathrm{ATP} \tag{15.9}$$

Stoichiometrically, only one molecule of ATP can be synthesized per electron pair from the oxidation of two Fe^{2+} when passed to oxygen at 100% efficiency. Because, as already discussed, the efficiency is much less than 100%, much ferrous iron needs to be oxidized to meet the energy demand of the *T. ferrooxidans* cell.

For a discussion of a molecular biochemical analysis of the ATPase of *T. ferrooxidans*, the reader is referred to Rawlings and Kusano (1994).

Reverse Electron Transport. The assimilation of CO_2 by any autotroph requires a source of reducing power. When *T. ferrooxidans* grows on iron, this source is Fe^{2+}. Ferrous iron thus has a dual function in the nutrition of this organism, namely as a source of energy and as a source of reducing power. To reduce fixed CO_2, electrons from Fe^{2+} are shunted to $NADP^+$ via the cytochrome system against an electropotential gradient by expenditure of energy (consumption of ATP) in a process called **reverse electron transport** (Aleem et al., 1963; Ingledew, 1982). The expenditure of energy is necessary because the cytochrome c_{oxd}/cytochrome c_{red} couple (the level at which electrons from iron enter the membrane-bound electron transport system) has a much higher $E_h^{\circ\prime}$ (+245 mV) than does the NAD/NADH couple ($E_h^{\circ\prime} = -320$ mV) or the NADP/NADPH couple ($E_h^{\circ\prime} = -324$ mV). In one strain of *T. ferrooxidans*, cytochrome c,

cytochromes c_1 and b (bc_1 complex), and a flavin were identified as participants in the reverse electron transport system (Tikhonova et al., 1967; Elbehti et al., 2000). As expected, arsenate, an uncoupler of oxidative phosphorylation, and amytal, an inhibitor of flavin reduction, blocked this system.

Carbon Assimilation. The major mechanism of CO_2 assimilation in *T. ferrooxidans* involves the Calvin–Benson cycle (Din et al., 1967b; Gale and Beck, 1967; Maciag and Lundgren, 1964). A minor CO_2 fixation mechanism involving phosphoenolpyruvate carboxylase also exists in the organism (Din et al., 1967b). The latter enzyme is needed for the formation of certain amino acids. In the Calvin–Benson cycle, CO_2 is fixed by ribulose 1,5-bisphosphate obtained from ribulose 5-phosphate as follows (see also Chap. 6).

$$\text{Ribulose 5-phosphate} + \text{ATP} \xrightarrow{\text{phosphoribulokinase}} \text{ribulose 1,5-bisphosphate} + \text{ADP} \quad (15.10)$$

$$\text{Ribulose 1,5-bisphosphate} + CO_2 \xrightarrow[\text{carboxylase}]{\text{Ribulose bisphosphate}} 2(\text{3-phosphoglycerate}) \quad (15.11)$$

Each 3-phosphoglycerate is then reduced to 3-phosphoglyceraldehyde:

$$\text{3-Phosphoglcycerate} + \text{NADPH} + H^+ + \text{ATP} \xrightarrow{\text{3-PGA dehydrogenase}} \text{3-phosphoglyceraldehyde} + NADP^+ + \text{ADP} + P_i \quad (15.12)$$

The 3-phosphoglyceraldehyde is converted in a series of steps to various cell constituents as well as to catalytic amounts of ribulose 5-phosphate to keep the Calvin–Benson cycle operating.

Phosphoenolpyruvate carboxylase catalyzes the fixation of CO_2 by phosphoenolpyruvate, which is formed from 3-phosphoglycerate as follows:

$$\text{3-Phosphoglycerate} \xrightarrow{\text{phosphoglyceromutase}} \text{2-phosphoglycerate} \quad (15.13)$$

$$\text{2-Phosphoglycerate} \xrightarrow{\text{enolase}} \text{phosphoenolpyruvate} \quad (15.14)$$

The phosphoenolpyruvate is then combined with CO_2:

$$\text{Phosphoenolpyruvate} + CO_2 \xrightarrow{\text{PEP carboxylase}} \text{oxalacetate} + P_i \quad (15.15)$$

Whether a functional tricarboxylic acid (TCA) cycle exists in *T. ferrooxidans* when it is growing autotrophically on iron is still unclear. Anderson and Lundgren (1969) reported experimental evidence for it in iron-grown cells, but Tabita and Lundgren (1971b) found it only in glucose-grown cells. The strain used in these studies may have been mixed with a satellite organism.

Thiobacillus prosperus

The mesophilic organism *Thiobacillus prosperus* resembles *T. ferrooxidans* closely except that it can grow in the presence of up to 6% NaCl (Huber and Stetter, 1989). Chloride ion is toxic to *T. ferrooxidans*.

Leptospirillum ferrooxidans

General Traits. *Leptospirillum ferrooxidans* was first isolated by Markosyan (1972) and was studied further by Balashova et al. (1974) and Pivovarova et al. (1981). A comparison with other acidophiles from the same habitat as *L. ferrooxidans* was first published by Harrison (1984). The original isolation was from a copper deposit in Armenia. It is an acidophilic organism. Its cells are vibrioid in shape with a polar flagellum about 25 nm in diameter. Involution cells may have a spiral shape. Tori form at pH values below 2. In the laboratory, organisms have been grown in Leten's (Leathen's) medium at pH 2–3 (Kuznetsov and Romanenko, 1963) and in 9 K medium (Silverman and Lundgren, 1959a) at pH 1.5. The organism oxidizes ferrous iron for energy but cannot oxidize reduced forms of sulfur and is incapable of growth in organic media; i.e., it is an obligate autotroph. At least one strain of *L. ferrooxidans*, CF12, oxidizes iron much more effectively at pH 1.25 and exhibits a lower temperature optimum ($\leq$25°C) than *T. ferrooxidans* ATCC 23270 ($\sim$30°C) (Gómez et al., 1999). However, two other strains of *L. ferrooxidans*, DSM 2705 and BC, have been shown to grow optimally at $\sim$32 and $\sim$35°C, respectively (Norris, 1990). The mechanism by which *L. ferrooxidans* oxidizes ferrous iron also differs from that of *T. ferrooxidans*. Its iron-oxidizing system lacks rusticyanin. Strain DSM 2705 of *L. ferrooxidans* contains a novel red cytochrome that is soluble, acid-stable, and reducible by ferrous iron and seems to lack an a-type cytochrome (Hart et al., 1991). It consists of a single polypeptide having a molecular mass of 17,900 Da and a standard reduction potential of +680 mV at pH 3.5. It contains 1 equivalent each of Fe and Zn (Hart et al., 1991). Strain P3A, on the other hand, contains a red cytochrome that has a smaller apparent molecular mass, 12,000 Da. This is in keeping with the conclusion of Harrison and Norris (1985) that not all morphologically similar acidophilic iron oxidizers called *Leptospirillum* belong to the same species. A similar conclusion was reached by Johnson et al. (1989) and González-Toril et al. (1999), who used a different experimental approach. Ferrous iron oxidation by *L. ferrooxidans*-like organisms is less susceptible to product inhibition by Fe^{3+} than that by *T. ferrooxidans* (Norris et al., 1988).

The cells of *L. ferrooxidans* have been shown to contain an active ribulose 1,5-bisphosphate carboxylase, typical of chemolithotrophs (autotrophs), which use the Calvin–Benson cycle for CO_2 assimilation (Balashova et al., 1974).

Metallogenium

An acid-tolerant organism, named *Metallogenium*, was reported from mesoacidic iron-bearing groundwaters (Walsh and Mitchell, 1972). It is a filamentous organism consisting of branching filaments (0.1–0.4 μm and >1 μm long) that are usually encrusted with iron oxide. The organism tolerates a pH range of 3.5–6.8, with an optimum at 4.1, and is thus intermediate between acidophiles and neutrophiles. In the laboratory, the addition of 0.024 M phthalate at pH 4.1 was important for observing iron oxidation and growth at initial Fe^{2+} concentrations greater than 100 mg L^{-1}. Acetate, citrate, or phosphate could not replace the phthalate in the medium. The growth medium contained $(NH_4)_2SO_4$, 0.1%; KH_2PO_4, 0.001%; $CaCO_3$, 0.01%; $MgSO_4 \cdot 7H_2O$, 0.02%; and $FeSO_4 \cdot 7H_2O$, 9%. These ingredients were dissolved in distilled water and the solution adjusted to pH 4.1. It is not clear whether this organism is an autotroph or a heterotroph. Care has to be taken in identifying it. Some inorganic precipitates may resemble it morphologically (Ivarson and Sojak, 1978).

Ferromicrobium acidophilum

Unlike the three previously presented iron-oxidizing mesophiles that are autotrophic, a unicellular iron-oxidizing mesophile that is obligately heterotrophic has been reported (Johnson and Roberto, 1997). It requires the presence of yeast extract to grow on iron. Its physiological temperature range is < 20–40°C (optimum 37°C). Under limited aeration, it also has the ability to reduce ferric to ferrous iron. The GC ratio in its DNA is 51–55 mol%. The type strain is T-23, and it has been tentatively named *Ferromicrobium acidophilum* (Johnson and Roberto, 1997; private communication, 2000).

Strain CCH7

Another acidophilic, heterotrophic, iron-oxidizing bacterium, as yet unnamed, was isolated by Johnson et al. (1993). One strain was labeled CCH7. It grew in a pH range of 2–4.4 (optimum pH 3.0). It formed streamers (filaments) more than 100 μm long when growing in liquid medium. The streamers broke up into motile cells and short filaments in later stages of growth. The authors concluded that the organism did not use Fe^{2+} as an energy source because addition of Fe^{2+} to the organic growth medium did not stimulate growth. The organism produced a sheath like the *Sphaerotilus-Leptothrix* group (see below), but its GC content of 62 mol% was lower than that of the *Sphaerotilus-Leptothrix* group, and it was acidophilic instead of neutrophilic.

Domain Bacteria: Thermophiles

Sulfobacillus thermosulfidooxidans

Sulfobacillus thermosulfidooxidans was isolated by Golovacheva and Karavaiko (1978) and further described by Norris (1990). It is a gram-positive, nonmotile, spore-forming (most strains), rod-shaped bacterium with tapered ends (0.6–0.8 × 1.0–1.3 μm, sometimes as long as 6 μm). The GC content of its DNA is 53.6–53.9 mol%. Its temperature range for growth is 28–60°C, with an optimum around 50°C, making it a *moderate thermophile*. It grows autotrophically on Fe(II), S^0, or metal sulfides as energy source and heterotrophically in the absence of an appropriate inorganic energy source (Golovacheva and Karavaiko, 1978). Autotrophic growth is stimulated by air enriched in CO_2 (Norris, 1997). It cannot use sulfate as a source of sulfur and consequently must be supplied with reduced sulfur (Norris and Barr, 1985). Autotrophic growth is stimulated by a trace (0.01–0.05%) of yeast extract (Golovacheva and Karavaiko, 1978), which causes the cells to increase significantly in size (Norris, 1997), but 0.1% yeast extract is inhibitory. Its pH range for growth is 1.9–3.0, the optimum range being 1.9–2.4 (Golovacheva and Karavaiko, 1978). A strain of this organism was formerly designated BC_1 (Norris, 1997).

Sulfobacillus acidophilus

Sulfobacillus acidophilus is closely related to *Sulfobacillus thermosulfidooxidans*. A strain of *S. acidophilus* was formerly designated as ALV (Norris et al., 1988). The two *Sulfobacillus* species differ in their GC content, with *S. thermosulfidooxidans* exhibiting a ratio of 48–50 mol% and *S. acidophilus* 55–57 mol% (Norris, 1997). Unlike the cells of *S. thermosulfidooxidans*, its cells do not increase significantly in size when growing on iron in the presence of a trace of yeast extract or when growth is heterotrophic in the absence of iron. Both species can appear elongate or in chains during autotrophic growth on mineral sulfides at acidities near maximum tolerance. In laboratory culture, *S. acidophilus* oxidizes elemental sulfur more readily than *S. thermosulfidooxidans*, whereas the latter oxidizes iron and mineral sulfides more readily (see Norris, 1997).

Acidimicrobium ferrooxidans

Acidimicrobium ferrooxidans is another moderately thermophilic bacterium that grows autotrophically when oxidizing ferrous iron in air (Norris, 1997). Two strains have been studied: TH3 (Norris et al., 1980) and ICP (Clark and Norris, 1996). The DNA of strain TH3 exhibits a GC ratio of 68.0 mol%, and that of strain ICP, 67.3 mol% (Clark and Norris, 1996). Strain TH3 can exhibit a filamentous growth habit (Clark and Norris, 1996). Growth of *A. ferrooxidans*

is not stimulated in air enriched with CO_2. It is less tolerant to ferric iron accumulation than *Sulfobacillus* spp. The ribulose bisphosphate carboxylase of *A. ferrooxidans* has an amino acid sequence that shares a high degree of similarity with that from *Thiobacillus ferrooxidans* (Norris, 1997).

Domain Archaea: Mesophile

Ferroplasma acidiphilum

Ferroplasma acidiphilum is an iron-oxidizing, chemolithotrophic, acidophilic microorganism whose cells are delimited by only a single peripheral membrane (Golyshina et al., 2000). Its pH optimum for growth is pH 1.7 (pH range 1.3–2.2). It requires a trace of yeast extract (0.02% in its growth medium).

Ferroplasma acidarmanus

Ferroplasma acidarmanus (Fig. 15.5) resembles *F. acidiphilum* but has a pH optimum of. 1.2 (pH range 0–2.5) and grows three times as fast as the latter at optimum pH. It has been found in biofilms in pyritic sediments in drainage tunnels at the Iron Mountain pyrite mine in California (Edwards et al., 2000). Like *F. acidiphilum*, it requires 0.02% yeast extract in its medium for autotrophic

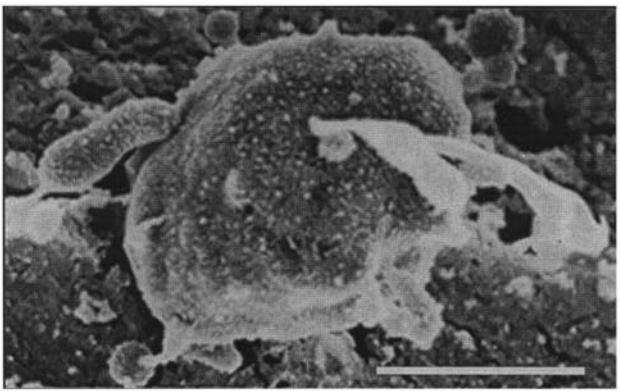


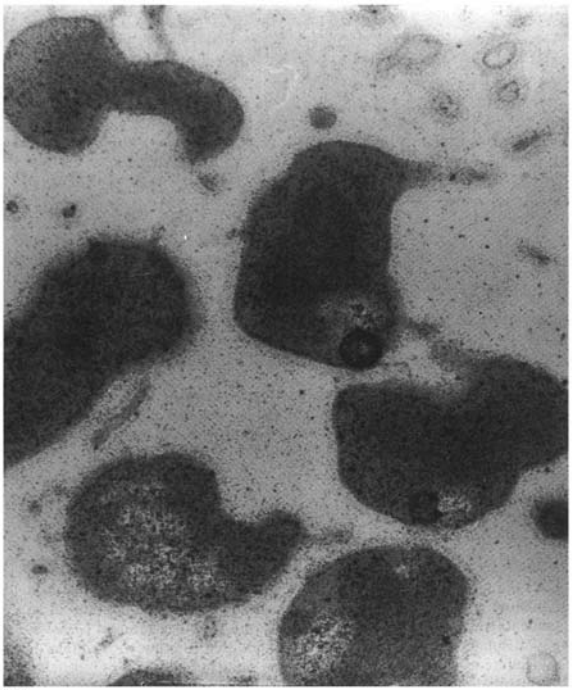
FIG. 15.5 A cryoscanning electron photomicrograph of *Ferroplasma acidarmanus* isolate fer1 (bar = 500 nm). The cells were in a late log growth phase when they were prepared for cryoscanning electron microscopy as described by Barker et al. (1998). The cells were viewed with a Hitachi S900 scanning electron microscope operated at 2 kV, on a Gatan cryostage. Irregularly sized and shaped cellular protrusions are inferred to be budding sections of the cell. (From Edwards KJ, Bond PL, Gihring TM, Bonfield JF, An archeal iron-oxidizing extreme acidophile important in acid mine drainage. Copyright 2000 by the American Association for the Advancement of Science, with permission.)

growth. It is also able to grow heterotrophically with yeast extract as sole energy source.

Domain Archaea: Thermophiles

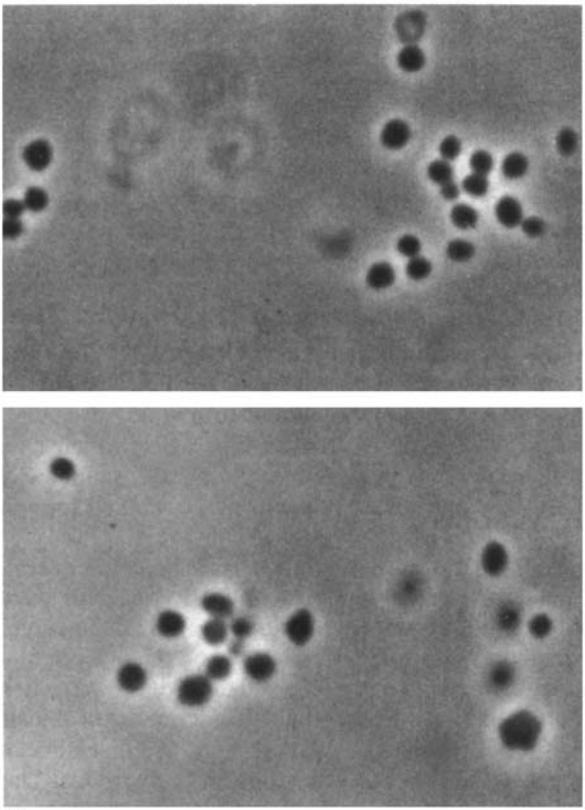
Acidianus brierleyi

Acidianus brierleyi (formerly *Sulfolobus*) *brierleyi* (Segerer et al., 1986) is an extremely thermophilic acidophile growing in the temperature range of 55–90°C (optimum 70–75°C) and a pH range of 1–5 (pH optimum ~3.0). It is a non-spore-forming, nonmotile, pleomorphic organism (1–1.5 μm in diameter) (Brierley and Brierley, 1973; Brierley and Murr, 1973; McClure and Wyckoff, 1982; Segerer et al., 1986) (Fig. 15.6A). The GC content of its DNA is 31 mol% (Segerer and Stetter, 1998). The organism contains no peptidoglycan in its cell



(A)

FIG. 15.6 Iron- and sulfur-oxidizing archaea. (A) *Acidianus* (formerly *Sulfolobus*) sp. (×28,760). (Courtesy of J. A. Brierley and C. L. Brierley.) (B) *Sulfolobus acidocaldarius* (×3540). (Reproduced from Brock TD, Brock KM, Belly RT, Weiss RL, *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch Mikrobiol 84: 54–68. Copyright 1972, by Springer-Verlag, with permission.)



(B)

FIG. 15.6 Continued.

wall nor does it feature an outer membrane as in gram-negative bacteria (Berry and Murr, 1980). Instead the cells are surrounded by a protein layer called the S-layer (Taylor et al., 1982). It can grow autotrophically on iron or S^0 as energy source. Autotrophic growth is stimulated by the addition of 0.2% yeast extract. CO_2 is not assimilated by a Calvin–Benson cycle but more likely by a reverse tricarboxylic acid cycle (Atlas, 1997) (see also Chap. 6). It can grow heterotrophically on yeast extract at elevated concentration.

Sulfolobus acidocaldarius

Sulfolobus acidocaldarius (Fig. 15.6B) is an extremely acidophilic, thermophilic, chemolithotrophic microorganism that has the capacity to use ferrous iron oxidation as a source of energy (Brock et al., 1972, 1976). It closely resembles

Acidianus brierleyi morphologically and physiologically. However, it differs from *A. brierleyi* on a genotypic basis (Segerer and Stetter, 1998). The GC content of its DNA is 60–68 mol%.

Domain Bacteria: Neutrophilic Iron Oxidizers

Gallionella ferruginea

Gallionella ferruginea is a mesophilic organism that consists of a bean-shaped cell and a lateral stalk of twisted bundles (Fig. 15.7) (see also figures in Hanert, 1981, and Ghiorse, 1984). The organism was first described by Ehrenberg (1836). Cholodny (1924) was the first to recognize that the bean-shaped cell on the lateral stalk was an intergral part of the organism. The stalk may branch dichotomously, each branch carrying a bean-shaped cell at its tip. The stalk is usually anchored to a solid surface. The stalk may be heavily encrusted with ferric hydroxide. The cells, which may form one or two polar flagella, may detach from their stalk, swim away as swarmers, seek a new site for attachment, and develop a stalked growth habit.

Gallionella has been described as a *gradient organism*; i.e., it grows best under low oxygen tension (0.1–1 mg O_2 L^{-1}) and in an E_h range of +200 to +320 mV (Kucera and Wolfe, 1957; Hanert, 1981). Its optimal growth temperature is 20°C (Hallbeck and Pedersen, 1990), although in nature the growth of some strains has been observed up to 47°C (Hanert, 1981). It prefers a pH range of 6.0–7.6 (Hanert, 1981). Its low oxygen requirement explains why this organism can catalyze Fe^{2+} oxidation at neutral pH. Under these partially reduced conditions, ferrous iron autoxidizes only slowly (Wolfe, 1964).

Hallbeck and Pedersen (1990) studied a strain of *G. ferruginea* in the laboratory whose cells when growing exponentially under aerobic gradient conditions were free-living and motile with a single flagellum and no stalk. Stalks began to form only in stationary phase when the cell population exceeded 6×10^5 mL^{-1} at a pH above 6. No stalks were formed when ferrous iron did not autoxidize under microaerobic conditions (e.g., pH 6.5, $E_h < -40$ mV). The investigators suggested that stalk formation by *G. ferruginea* protects against "increasing reducing capacity of ferrous iron as it becomes unstable in an environment that becomes oxidizing."

Gallionella can grow autotrophically and mixotrophically. Hallbeck and Pedersen (1991) demonstrated that their organism obtained all its carbon from CO_2 when growing in a mineral salts medium in an aerobic gradient with ferrous sulfide as energy source. The same investigators showed that glucose, fructose, and sucrose could meet part of the energy requirement and part or all of the carbon requirement of the organism, depending on the concentration of sugars. Hanert (1968) previously claimed that *Gallionella* does not grow without

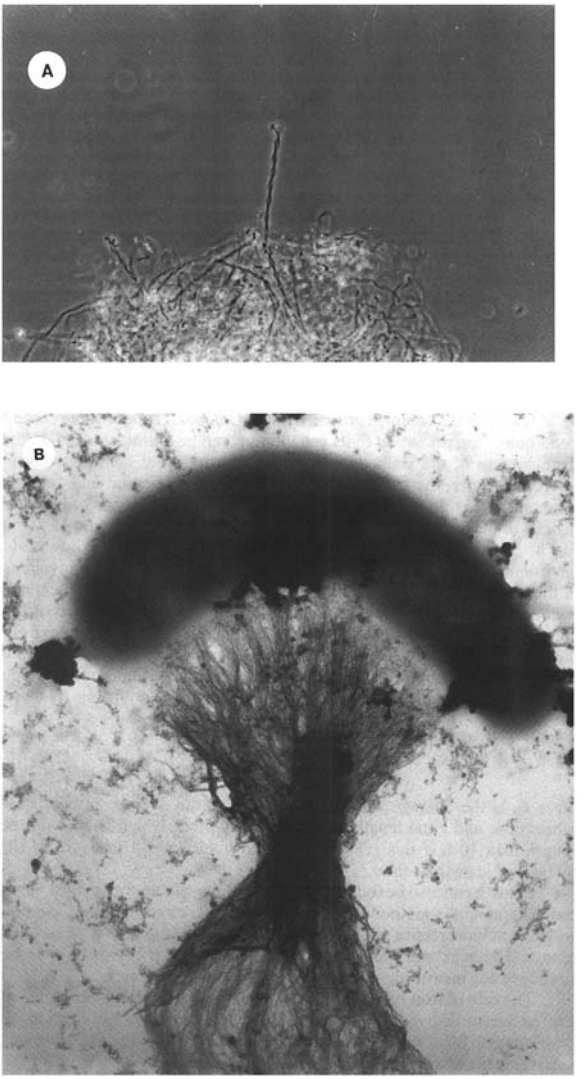


FIG. 15.7 (A) Phase contrast photomicrograph of a tangle of *Gallionella ferruginea*. Note the small cell at the tip of the twisted stalk projecting from the tangle (×576). (B) Electron photomicrograph of unstained and unshadowed *Gallionella* cell showing fibrillar nature of lateral twisted stalk (×65,160). (Courtesy of W. C. Ghiorse.)

oxidizable iron in the medium. It may well be that strictly autotrophic as well as facultatively autotrophic strains exist. The organism appears to fix CO_2 via ribulose 1,5-bisphosphate carboxylase/oxidase (Lütters and Hanert, 1989; Hallbeck and Pedersen, 1991).

According to Lütters-Czekalla (1990), *G. ferruginea* strain BD was able to grow using sulfide and thiosulfate as energy sources and electron donors but not elemental sulfur or tetrathionate at the interface of the oxidizing and reducing zones of a microgradient culture. Addition of organic carbon did not stimulate growth. Under these growth conditions, the organism did not form the characteristic stalk it forms when growing on Fe(II). Luetters-Czekalla (1990) took this to indicate that the stalk is a product of iron oxidation. Her culture nevertheless excreted a significant amount of an unidentified extracellular polymeric material. The strain of *G. ferrooxidans* isolated by Hallbeck (1993) was not able to grow with sulfide or thiosulfate as sole source of energy and reducing power.

Isolation and propagation methods for *Gallionella* were summarized by Hanert (1981). They are adaptations of the method originally described by Kucera and Wolfe (1957), using the medium formulated by them. The medium consists of NH_4Cl, 0.1%; K_2HPO_4, 0.05%; and $MgSO_4$, 0.02%; with freshly prepared ferrous sulfide suspension making up 10% of the total volume of medium. Tap water or natural water is used in making up the medium because distilled water apparently does not supply a required component. A small amount of CO_2 is bubbled through the salts solution prior to the addition of ferrous sulfide. The medium is placed in test tubes, which are stoppered to prevent loss of CO_2. A redox gradient is established in the culture medium as oxygen from the air diffuses into it and reacts with some of the FeS. When growing in this medium, *Gallionella* occupies the lower two-thirds of the volume above the ferrous sulfide. The organism will not grow anaerobically, whether nitrate is added or not.

Owing to the complex growth habit of *Gallionella* under some culture conditions, determination of its growth rate can be a problem. Individual development of the stalked organism can be followed quantitatively in microculture by microscopically following stalk elongation and twisting (Hanert, 1974a). In other words, growth is measured in terms of increase in mass of the organism. An elongation rate of 40–50 $\mu m\, h^{-1}$ in the first generation has been obtained. This is two to four times as fast as in a natural environment. Stalks do not elongate further after three or four divisions of the apical cell. Stalk lengthening occurs at the tip where the apical cell is attached. The stalk twists as it lengthens, owing to the rotation of the apical cell. The rotation occurs at a constant rate. In a natural environment, the rate of growth of *Gallionella* is measured in terms of the rate of attachment to a solid surface such as a submerged microscope slide, and the rate of stalk elongation is shown in the relationship (Hanert, 1973)

$$V_t = \frac{b_v l_v}{2} t^2 \tag{15.16}$$

Here b_v is the average rate of attachment, l_v the average rate of stalk elongation, and t the length of the growth period, which should not be longer than 10 hr if this relationship is to hold. V_t is a measure of the amount of growth at time t.

Growth can also be followed by determining viable counts by a most probable numbers method and total counts by a direct counting method with an epifluorescence microscope after staining the cells with acridine orange (Hallbeck and Pedersen, 1990). These latter methods have to be employed for measuring growth when no stalks are formed.

The rate of iron oxidation by *Gallionella* in the natural environment may be measured by submerging a microscope slide at a site of *Gallionella* development for a desired length of time, then removing the slide and measuring the amount of iron deposited on it (Hanert, 1974b). The quantity of iron deposited can be expressed in terms of the amount of iron per unit surface area of the slide that was submerged and on which iron was laid down.

Sheathed, Encapsulated, and Wall-Less Iron Bacteria

Other mesophilic bacteria that have been associated with ferrous iron oxidation at circumneutral pH include sheathed bacteria (Fig. 15.8) such as *Sphaerotilus*, *Leptothrix* spp., *Crenothrix polyspora*, *Clonothrix* sp., and *Lieskeella bifida*, some encapsulated bacteria like the Siderocapsaceae group, and three as yet unnamed strains of microaerobic iron oxidizers, strains ES-1, ES-2, and RL-1 (Emerson, 2000). Many of these are more likely iron-depositing bacteria, i.e., they bind preoxidized iron at their cell surface (Ghiorse, 1984; Ghiorse and Ehrlich, 1992).

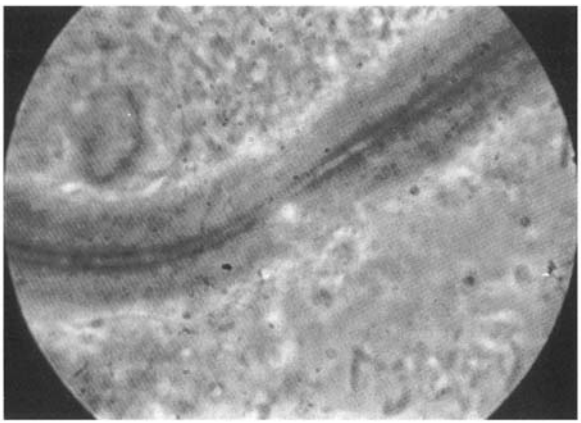


FIG. 15.8 *Leptothrix* sp. Portion of the sheathed organism (×3460). (Courtesy of E. J. Arcuri.)

Ultrastructural examination of the sheath of *Leptothrix discophora* SP-6 showed it to be a tubular structure of condensed fibrils (6.5 nm in diameter) overlain by a somewhat diffuse capsular layer (Emerson and Ghiorse, 1993a). The fibrillar part of the sheath was anchored by bridges to the outer membrane of the gram-negative cells in the sheath. The capsular layer had a net negative charge. Purified sheaths contained 34–35% polysaccharide consisting of a 1 : 1 mixture of uronic acids and galactosamine, 23–25% protein enriched in cysteine, 8% lipid, and 4% inorganic ash. The cysteine in the sheath protein is thought to be important in the maintenance of integrity of the sheath (Emerson and Ghiorse, 1993b).

Leptothrix spp., which have sometimes been classed with *Sphaerotilus* (Pringsheim, 1949; Stokes, 1954; Hoehnl, 1955), has been examined on several occasions for enzymatic iron oxidation. Winogradsky (1888) first reported that *L. ochracea* (probably *L. discophora*, according to Cholodny, 1926) could oxidize ferrous iron. He found that he could grow the organism in hay infusion only if he added ferrous carbonate. The iron was oxidized and deposited in the sheath of the organism. He inferred from this observation that the organism was an autotroph. Molisch (1910) and Pringsheim (1949) disagreed with Winogradsky's conclusion about iron oxidation by *L. ochracea*, believing that the organism merely deposited autoxidized iron in its sheath. However, Lieske (1919) confirmed Winogradsky's observations of growth on ferrous carbonate in very dilute organic solution and suggested that the organism might be mixotrophic. Cholodny (1926), Sartory and Meyer (1947), and Praeve (1957) also made observations similar to those of Winogradsky and Lieske. Most claims in the past for enzymatic iron oxidation by *Leptothrix* were based mainly on the growth requirement for ferrous iron in dilute medium and on the oxidation of the ferrous iron during growth. However, Praeve (1957) also showed a stimulation of oxygen uptake by the organism when Fe^{2+} was present as the only exogenous, oxidizable substrate in Warburg respiration experiments. Significantly, he found that empty sheaths were unable to take up oxygen on Fe^{2+}. Most recently, de Vrind-de Jong et al. (1990) reported iron-oxidizing activity in spent medium from a culture of *L. discophora* SS-1. Corstjens et al. (1992) related this iron-oxidizing activity to a 150 kDa protein. It behaved like an enzyme and was not produced by a spontaneous mutant strain that lacked iron-oxidizing activity. The factor was distinct from the manganese-oxidizing protein excreted by the wild-type SS-1 strain described by Adams and Ghiorse (1987).

Enzymatic iron oxidation by all other sheathed bacteria is at most presumptive, based on gross morphological similarities with *Leptothrix* and the observation of oxidized iron deposits on their sheaths. It is quite likely that most or all of these organisms merely deposit preoxidized iron on their sheaths.

Dubinina (1978a, 1978b) has reported that *L. pseudoochracea*, *Metallogenium*, and *Arthrobacter siderocapsulatus* oxidized Fe^{2+} with metabolically produced H_2O_2 through catalysis by catalase of the organism.

A wall-less bacterium, *Mycoplasma laidlawii* (now known as *Acholeplasma laidlawii*) has been reported to oxidize ferrous iron (Balashova and Zavarzin, 1972). The organism was cultured in a salt-free meat–peptone medium containing iron wire or powder. Ferric iron was formed during active growth and, in part, precipitated on the cells of the organism. Addition of catalase was found to depress ferric oxide production, suggesting that H_2O_2 played a role in the oxidation process. It is interesting that in this instance catalase did not accelerate iron oxidation as was found by Dubinina with other organisms (Dubinina, 1978a). It is not clear from the report whether other enzymes played a direct role in the oxidation of iron in this instance.

It seems that different mechanisms of enzymatic iron oxidation exist among neutrophilic bacteria.

15.5 ANAEROBIC OXIDATION OF FERROUS IRON

Although until recently bacterial oxidation of ferrous iron was generally assumed to require oxygen as terminal electron acceptor, exceptions that occur at circumneutral pH have been found. Two anaerobic photosynthetic bacteria resembling *Rhodomicrobium vannielii* and *R. palustris*, repectively, and one strain, resembling *Thiodictyon* spp. morphologically, were shown to oxidize Fe(II) to Fe(III) oxides in the light. These bacteria used the reducing power generated in the oxidation of Fe(II) to Fe(III) oxide in CO_2 assimilation (fixation) (Ehrenreich and Widdel, 1994; Widdel et al., 1993). No iron oxidation was observed anaerobically in the dark or in the light in the absence of the bacteria. Furthermore, no growth by these bacteria occurred in the test medium in the absence of added iron. Widdel et al. (1993) suggested that this anaerobic oxidation of ferrous to ferric iron by phototrophic bacteria may have contributed to the early stages of Banded-Iron Formations that arose in Archean times.

In addition to anaerobic iron-oxidizing phototrophs, respirers that oxidize Fe(II) anaerobically have been described. Straub et al. (1996) found that enrichment cultures from town ditches in Bremen, Germany, and from brackish water lagoons, and some denitrifying isolates were able to oxidize iron anaerobically in pure culture using nitrate as terminal electron acceptor. Some were able to use ferrous iron as exclusive electron donor and grew lithotrophically, and some used acetate concurrently with ferrous iron as electron donors and thus grew mixotrophically. The nitrate was reduced to dinitrogen in all instances. Ammonia production from nitrate was not detected. *Thiobacillus denitrificans* was also found to be able to reduce nitrate with ferrous iron (Straub et al., 1996).

The following equation summarizes the bio-oxidation of ferrous iron by nitrate in these observations (Straub et al., 1996):

$$10FeCO_3 + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 10HCO_3^- + 8H^+ \quad (15.17)$$

Benz et al. (1998) detected anaerobic, nitrate-coupled ferrous iron oxidation in culture enrichments with sediments from freshwater, brackish water, and marine water. They isolated a strain labeled HidR2, which was a motile, non-spore-forming, gram-negative rod that oxidized ferrous iron in the presence of 0.2–1.1 mM acetate as cosubstrate and nitrate as terminal electron acceptor at pH 7.2 and 30°C. The ferrous iron served as energy source. Although the authors identified this strain as heterotrophic, its growth with ferrous iron as energy source indicates that it grows mixotrophically under these conditions. The organism is capable of anaerobic growth on acetate in the absence of ferrous iron but in the presence of nitrate. It is also capable of aerobic growth in the presence of low concentrations of acetate with O_2 as terminal electron acceptor.

A hyperthermophilic archeon, *Ferroglobus placidus*, has also been found to oxidize ferrous iron anaerobically with nitrate as electron acceptor (Hafenbrandl et al., 1996). It can grow lithoautotrophically or heterotrophically between 65 and 95°C (optimum 85°C) and at pH 7.0. The cells require 0.5–4.5% NaCl (~2% optimum) in their growth medium to prevent their lysis. The organism reduces the nitrate chiefly to nitrite, but during prolonged incubation the nitrite is converted to NO and NO_2. No N_2, N_2O, or NH_3 is formed, however. The following reaction best describes the overall oxidation of ferrous iron by this organism (Hafenbrandl et al., 1996, amended):

$$2FeCO_3 + NO_3^- + 5H_2O \rightarrow 2Fe(OH)_3 + NO_2^- + 2HCO_3^- + 2H^+ \quad (15.18)$$

The bacterial processes described in this section can be considered to be part of an anaerobic iron cycle.

15.6 IRON(III) AS TERMINAL ELECTRON ACCEPTOR IN BACTERIAL RESPIRATION

Ferric iron in nature may be microbiologically reduced to ferrous iron. The ferric iron that is reduced by microbes may be in dissolved or insoluble form. Examples of insoluble forms are amorphous oxides or hydroxides, minerals like limonite (FeOOH), goethite ($Fe_2O_3 \cdot H_2O$), hematite (Fe_2O_3), and so forth. As in the case of iron oxidation, this reduction may be enzymatic or nonenzymatic. Enzymatic ferric iron reduction may manifest itself as a form of respiration, mostly anaerobic, in which ferric iron serves as *dominant* or *exclusive* terminal electron

acceptor, or it may accompany fermentation, in which ferric iron serves as a *supplementary*, as opposed to dominant or exclusive, terminal electron acceptor. Both of these ferric iron–reducing processes are forms of *dissimilatory iron reduction*.

When ferric iron is reduced during uptake for incorporation into specific cellular components, the process represents *assimilatory iron reduction*. Relatively large quantities of iron are consumed in dissimilatory reduction, whereas only very small quantities are consumed in assimilatory reduction. The ferric iron in assimilatory reduction when acquired at circumneutral pH is usually complexed by siderophores and may be reduced in this complexed form or after release from the ligand in the cell envelope (discussed earlier).

The emphasis in the following sections will be on dissimilatory iron reduction.

Bacterial Ferric Iron Reduction Accompanying Fermentation

For some time, ferric iron has been known to influence fermentative metabolism of bacteria as a result of its ability to act as terminal electron acceptor. Roberts (1947) showed a change in fermentation balance when comparing the action of *Bacillus polymyxa* on glucose anaerobically in the presence and absence of iron (Table 15.4). The ferric iron in these experiments was supplied as freshly precipitated, dialyzed ferric hydroxide suspension obtained in a reaction of ferric chloride and an excess of potassium hydroxide. The suspension had a pH of 7.8. The ferric iron seemed to act as a supplementary electron acceptor in fermentation and in this way changed the relative quantities of certain products formed from glucose. Thus, in the presence of iron, less H_2, CO_2, and 2,3-butylene glycol and more ethanol were formed in either organic or synthetic medium than in the absence of iron. Also, more glucose was consumed in the presence of iron than in its absence in either medium.

Bromfield (1954a) showed that besides *B. polymyxa*, growing cultures of *B. circulans* can also reduce ferric iron. Depending on the medium, he found that even *Escherichia freundii*, *Aerobacter* (now *Enterobacter*) sp., and *Paracolobactrum* (now probably *Citrobacter*) can do so. However, he inferred from his results that the reduction of iron was not directly involved in the oxidation of the substrate (energy source), which is at variance with his results with resting cells (Bromfield, 1954b). He found that completely anaerobic conditions were not required to obtain ferric iron reduction by bacteria. But when the level of aeration of the cultures was increased, ferrous iron became reoxidized due to autoxidation.

Bromfield (1954b) also showed that washed cells of *Bacillus circulans*, *B. megaterium*, and *Enterobacter aerogenes* reduced ferric iron of several ferric compounds [$FeCl_3$, $Fe(OH)_3$, $Fe(lactate)_2$] in the presence of such suitable

TABLE 15.4 Fermentation Balances for *Bacillus polymyxa* Growing in Two Different Media in the Presence and Absence of Ferric Hydroxide[a]

	Synthetic medium[b] (mol/100 mol glucose)		Organic medium[c] (mol/100 mol glucose)	
Products	$-Fe(OH)_3$	$+Fe(OH)_3$	$-Fe(OH)_3$	$+Fe(OH)_3$
CO_2	199	170	186	178
H_2	51	31	53	33
HCOOH	11	12	9	12
Lactic acid	17	19	14	7
Ethanol	72	82	78	94
Acetoin	0.5	1	1	2
2,3-Butylene glycol	64	51	49	44
Acetic acid	0	0	0	0
Iron reduced	—	42	—	61
Glucose fermented (mg/100 mL)	1029	2333	1334	2380
C recovery (%)	112.1	101.8	98.8	97.2
O/R index	1.06	1.0	1.06	1.03

[a] Incubation was for 7 days at 35°C.
[b] Glucose, 2.4%; asparagine, 0.5%; K_2HOP_4, 0.08%; KH_2PO_4, 0.02%; KCl, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.5%.
[c] Glucose, 2.5%; peptone, 1%; K_2HPO_4, 0.08%; KH_2PO_4, 0.02%; KCl, 0.02%; $MgSO_4 \cdot 7H_2O$, 0.5%.
Source: Roberts (1947), with permission.

electron donors as glucose, succinate, and malate. He was able to inhibit reduction by boiling the cells or by adding chloroform or toluene to the reaction mixture, but he did not observe inhibition with either azide or cyanide. He interpreted his findings to indicate that ferric iron reduction was associated with dehydrogenase activity. He thought, however, that the reduction of insoluble ferric iron (e.g., ferric hydroxide) could have occurred only in the presence of a complexing agent. From more recent studies, it has become clear that although some complexing agents may speed up the rate of reduction, as did α,α-dipyridyl in his experiments and those of De Castro and Ehrlich (1970), nitriloacetic acid in experiments of Lovley and Woodward (1996), or anthraquinone-2,6-disulfonate in experiments of Lovely et al. (1996, 1998) and of Nevin and Lovely (2000), they are not essential.

Some other bacteria have since been shown to be able to reduce ferric iron in conjunction with a fermentative process. They include aerobes such as *Pseudomonas* spp. and *Vibrio* sp., and anaerobes such as *Clostridium* spp. and *Bacteroides hypermegas* (see review by Lovley, 1987). The iron-reducing activity

was viewed in several of these instances as a sink for excess reducing power from which the organisms could not derive useful energy in its oxidation (see reviews by Lovley, 1987, 1991). However, this explanation holds only if it can be demonstrated that iron reduction in these instances is not accompanied by energy conservation. This was reported to be the case with *Clostridium beijerinckii* (Dobbin et al., 1999). *Pseudomonas ferrireductans* (now *Shewanella putrefaciens* strain 200) contains both constitutive and inducible ferric iron reductase. The constitutive enzyme is involved in ferric iron respiration (in which energy is conserved), and the inducible enzyme, produced at lower oxygen tension, is involved in electron scavenging without energy conservation (electron sink) (Arnold et al., 1986a).

Even some fungi have been implicated in Fe(III) reduction (e.g., Ottow and von Klopotek, 1969). However, their ability to reduce ferric iron is not likely to involve anaerobic respiration but instead either assimilatory iron reduction or the production of one or more metabolic products that act as a chemical reductant of the ferric iron.

Ferric Iron Respiration

Typical heterotrophic and autotrophic ferric iron respirers are listed in Table 15.5. The entries in this table show that the ability to use ferric iron as terminal electron acceptor is spread among a variety of members of the Bacteria and Archaea domains. These include strictly anaerobic and facultative organisms, the latter of which can grow aerobically as well as anaerobically. In general, all of them reduce Fe(III) only anaerobically, but a few exceptions are known (e.g., Short and Blakemore, 1986; De Castro and Ehrlich, 1970; Brock and Gustafson, 1976). The electron donors used by heterotrophic Fe(III) respirers include a wide range of organic compounds as well as H_2 (Lovley and Lonergan, 1990; Lovley et al., 1989a, 1989b; Coates et al., 1999). The organic compounds include substances as simple as acetate and lactate and as complex as palmitate and some aromatic compounds. Different Fe(III) reducers utilize different types of these compounds. Some of the organisms are unable to degrade their organic substrate completely, usually accumulating acetate (e.g., Lovley et al, 1989b). To serve as terminal electron acceptor in respiration of iron reducers, the ferric iron at circumneutral pH may be in the form of a soluble complex formed with citrate, nitrilotriacetate, or some other ligand (Lovley and Woodward, 1996). Various iron reducers have also been shown to attack insoluble forms of Fe(III) such as crystalline goethite (e.g., Roden and Zacchara, 1996; Nevin and Lovley, 2000). Ferrous iron, which is the product of Fe(III) reduction, when adsorbed at the surface of an iron oxide like goethite, interferes with microbial attack of the iron oxide. Its removal from the iron oxide surface promotes the reduction of the oxide (Roden and Urrutia, 1999; Roden et al., 2000).

As Table 15.5 shows, some autotrophs such as *Thiobacillus thiooxidans*, *T. ferrooxidans*, *Leptospirillum ferrooxidans*, *Sulfolobus* spp., *Sulfobacillus acidophilus*, *Sulfobacillus thermosulfidooxidans*, and *Acidimicrobium ferrooxidans*, can also respire with Fe(III) as terminal electron acceptor using S^0 as electron donor (Bridge and Johnson, 1998; Brock and Gustafson, 1976; Pronk et al., 1992; Sugio et al., 1992b). *T. thiooxidans* can bring this reduction about aerobically because the ferrous iron it produces at acid pH (around pH 2.5) does not autoxidize readily. On the other hand, *T. ferrooxidans* accumulates Fe^{2+} only anaerobically because aerobically it reoxidizes the Fe^{2+}. *Sulfolobus acidocaldarius* can form Fe^{2+} microaerophilically at 70°C because under limited oxygen availability it does not reoxidize Fe^{2+}. *Acidimicrobium ferrooxidans*, *Sulfobacillus acidophilus*, and *S. thermosulfidooxidans* also reduce Fe(III) to Fe^{2+} under oxygen limitation, but they perform this reduction best with organic electron donors (Bridge and Johnson, 1998). According to Corbett and Ingledew (1987), some growing cultures of *T. ferrooxidans* appear to use a branched pathway when oxidizing sulfur aerobically in the presence of ferric iron in which electrons from sulfite pass to iron(III) or O_2 via a cytochrome bc_1 complex. The reactions can be summarized as follows:

$$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e \quad (15.19)$$

$$2Fe^{3+} + 2e \rightarrow 2Fe^{2+} \quad (15.20)$$

$$0.5O_2 + 2H^+ + 2e \rightarrow H_2O \quad (15.21)$$

The sulfite is a metabolic intermediate in the oxidation of S^0. Its formation from sulfur involves an oxygenation and thus requires oxygen (see Chap. 18).

In contrast to observations with another strain of *T. ferrooxidans* (Brock and Gustafson, 1976), *T. ferrooxidans* AP10-3 reduces ferric iron both aerobically and anaerobically with sulfur by an enzyme system that includes a sulfide : Fe(III) and a sulfite : Fe(III) oxidoreductase (Sugio et al., 1989, 1992a). Other strains of *T. ferrooxidans* and of *Leptospirillum ferrooxidans*, which was not previously known to oxidize sulfur (S^0), also possess this enzyme system (Sugio et al., 1992b). In this process, the bacteria transform elemental sulfur to sulfide in the presence of reduced glutathione (GSH). The reactions involved in sulfur oxidation with Fe(III) by *T. ferrooxidans* AP19-3 can be summarized as follows:

$$S^0 + 2GSH \rightarrow H_2S + GSSG \quad (15.22)$$

$$H_2S + 6Fe^{3+} + 3H_2O \rightarrow SO_3^{2-} + 6Fe^{2+} + 8H^+ \quad (15.23)$$

$$SO_3^{2-} + 2Fe^{3+} + H_2O \rightarrow SO_4^{2-} + 2Fe^{2+} + 2H^+ \quad (15.24)$$

Growth on sulfur in the presence of Fe(III) by *T. ferrooxidans* AP19-3 occurs only aerobically (Sugio et al., 1988a, 1988b). *L. ferrooxidans* has so far not been grown on sulfur (Sugio et al., 1992b). It seems to conserve energy only

TABLE 15.5 Fe(III)-Respiring Bacteria

Organism	References
a. Heterotrophs: strict anaerobes	
Bacillus infernus	Boone et al., 1995
Clostridium spp.	See discussion in text.
Desulfobulbus propionicus	Lonergan et al., 1996
Desulfovibrio desulfuricans	Coleman et al., 1993
Desulfuromonas acetoxidans	Roden and Lovley, 1993
Desulfuromusa bakii	Lonergan et al., 1996
Desulfuromusa succinoxidans	Lonergan et al., 1996
Desulfuromusa kysingii	Lonergan et al., 1996
Ferribacterium limneticum	Cummings et al., 1999
Geobacter chapellii	Lonergan et al., 1996
Geobacter hydrogenophilus	Lonergan et al., 1996
Geobacter metallireducens (formerly strain GS-15)	Lovley et al., 1993
Geobacter sulfurreducens	Caccavo et al., 1994
Geospirillum barnesii[a]	Laverman et al., 1995; Lonergan et al., 1996
Geothrix fermentans	Coates et al., 1999
Geovibrio ferrireducens	Caccavo et al., 1996
Pyrobaculum islandicum	Huber et al., 1987; Kashefi and Lovley, 2000
P. acetylinicus	Lonergan et al., 1996
Pelobacter carbinolicus	Lonergan et al., 1996
Pelobacter propionicus	Lonergan et al., 1996
Pelobacter ventianus	Lonergan et al., 1996
b. Heterotrophs: facultative aerobes	
Aeromonas hydrophila	Knight and Blakemore, 1998
Bacillus spp.	See discussion in text.
Ferrimonas balearica	Rosselló-Mora et al., 1995
Pseudomonas sp.	Balashova and Zavarzin, 1979
Shewanella alga	Caccavo et al., 1992
Shewanella (formerly *Pseudomonas*, *Alteromonas*) *putrefaciens*	Obuekwe and Westlake, 1982; Arnold et al., 1986a, 1986b; Myers and Nealson, 1988; Lovley et al., 1989a
Shewanella sp.	Roselló-Mora et al., 1994
c. Autotrophs	
Acidimicrobium ferrooxidans	Bridge and Johnson, 1998
Leptospirillum ferrooxidans[b]	Sugio et al., 1992b
Sulfobacillus acidophilus	Bridge and Johnson, 1998
Sulfobacillus thermosulfidooxidans	Bridge and Johnson, 1998
Sulfolobus spp.	Brock and Gustafson, 1976
Thiobacillus ferrooxidans	Brock and Gustafson, 1976; Pronk et al., 1992
Thiobacillus thiooxidans	Brock and Gustafson, 1976

[a] Now *Sulfurospirillum barnesii*.
[b] No growth when reducing Fe(III) with sulfur (Sugio et al., 1992b).

from iron oxidation, not sulfur oxidation by ferric iron. Chemical oxidation of sulfur intermediates by ferric iron in the periplasm of *T. ferrooxidans* AP19-3 also occurs (Sugio et al., 1985). Sulfite oxidation by ferric iron in *T. ferrooxidans* AP19-3 is viewed as a mechanism of detoxification because sulfite is toxic if allowed to accumulate (Sugio et al., 1988b).

Dissimilatory iron reduction in the form of anaerobic respiration has now been recognized as an important means of mineralization of organic matter in environments in which sulfate or nitrate occurs in amounts insufficient to sustain sulfate or nitrate respiration, respectively (Lovley, 1987, 1991; Nealson and Saffarini, 1994). The process can operate with various organic acids, including volatile fatty acids, and with aromatic compounds as electron donors. It can displace methanogenesis by outcompeting for H_2 and acetate (Lovley, 1991).

Metabolic Evidence for Enzymatic Ferric Iron Reduction

Most of the early evidence for dissimilatory ferric iron reduction rested on observations with growing cultures. Troshanov (1968, 1969), following up on Bromfield's earlier observations, demonstrated that *Bacillus circulans*, *B. mesentericus*, *B. cereus*, *B. centrosporus*, *B. mycoides*, *B. polymyxa*, *Pseudomonas liquefaciens*, and *Micrococcus* sp., which he isolated from sediment from several lakes on the Karelian peninsula in the former U.S.S.R., could reduce ferric iron to varying degrees. He found that all his cultures that reduced ferric iron could also reduce manganese(IV), but the reverse was not true. The effect of oxygen on iron(III) reduction in his experiments depended on the culture he tested. Some organisms, such as *B. circulans*, reduced iron more readily anaerobically; others, including *B. polymyxa*, did not. Troshanov noted that the form in which the iron was presented to his cultures affected the rate of its reduction. Insoluble ferric iron in bog ore was reduced more slowly than soluble $FeCl_3$. Cultures also varied in their ability to reduce insoluble ferric iron at all. He found that *B. circulans* actively reduced bog iron ore whereas *B. polymyxa* did not.

Similar findings were made independently with soil bacteria by Ottow and collaborators (Ottow, 1969a, 1971; Ottow and Glathe, 1971; Hammann and Ottow, 1974; Munch et al., 1978; Munch and Ottow, 1980). Support for the notion that ferric iron reduction in these instances was enzymatic was gained from the observation that nitrate and chlorate can reversibly inhibit ferric iron reduction by members of the genera *Enterobacter* and *Bacillus* as well as *Pseudomonas* and *Micrococcus* (Ottow, 1969b, 1970a). Because all of these organisms possess dissimilatory nitrate reductase, which catalyzes reduction of nitrate and chlorate (Pichinoty, 1963), it was inferred that in these organisms iron reductase is the same enzyme as nitrate reductase. However, this is not the case in all iron reducers, because some other bacteria (e.g., *B. pumilus*, *B. sphaericus*,

Clostridium saccharobutylicum, and *C. butyricum*) that lack nitrate reductase activity can nevertheless reduce ferric iron (Ottow, 1970a; Munch and Ottow, 1977). The investigators inferred that these organisms must possess another kind of iron reductase. They supported this inference with observations with mutants that lacked nitrate reductase (Nit^-), which they derived from wild-type strains possessing the enzyme (Nit^+). Iron reduction with these Nit^- strains was found to be insensitive to inhibition by nitrate or chlorate (Ottow, 1970a). Most of the Nit^- mutants reduced iron less rapidly than did their wild-type parent, but an Nit^- mutant of *B. polymyxa* reduced iron more intensely than its wild-type parent. Also consistent with the inference that some dissimilatory iron reducers use an enzyme other than nitrate reductase in Fe(III) reduction, Ottow and Ottow (1970) noted that the size of the soil microflora capable of reducing iron is usually greater than the size of the microflora capable of reducing nitrate. The list of inhibitors of bacterial ferric iron reduction has been extended to include permanganate, dichromate, sulfite, thiosulfate, and the redox dyes methylene blue, indochlorophenol, and phenazine methosulfate when *Shewanella* (*Pseudomonas*) *putrefaciens* strain 200 is the test organism (Obuekwe and Westlake, 1982).

Nitrate inhibition of ferric iron reduction need not be due to competitive inhibition of iron reductase (nitrate reductase) (Obuekwe et al., 1981). Nitrate was found to stimulate ferric iron reduction by *S. putrefaciens* (*Pseudomonas* sp.) during short-term incubation but to depress it during long-term incubation. The inhibitory effect of nitrate in this instance was related to chemical oxidation of ferrous iron to ferric iron by nitrite (see also discussion by Sørensen, 1987). Nevertheless, the authors found that when *S. putrefaciens* was preinduced by nitrate, the resultant induced strain reduced Fe(III) faster than the uninduced strain, supporting the notion that nitrate reductase can catalyze ferric iron reduction by this organism. Obuekwe and Westlake (1982) explained this effect as merely reflecting a better physiological state of induced cells than of uninduced cells. Nitrate may also act as a noncompetitive inhibitor of ferric iron reduction, as in *Staphylococcus aureus* (Lascelle and Burke, 1978).

Other early evidence of the enzymatic nature of Fe(III) reduction by some microbes was the observation by De Castro and Ehrlich (1970) that a cell extract from marine *Bacillus* strain 29A, whose intract cells actively reduced ferric iron, could reduce iron(III) in the mineral limonite. This activity was partially destroyed by heating and inhibited by mercuric chloride and *para*-mercurybenzoate. Lascelle and Burke (1978) detected ferric iron reduction by a membrane fraction from *Staphylococcus aureus*, which could also reduce nitrate. They found evidence for involvement of a branched electron transport pathway in ferric iron reduction in their organism. By use of selective inhibitors, they showed that nitrate received electrons via a cytochrome b–requiring branch whereas ferric iron received electrons via a branch that originated ahead of cytochrome b. Nitrate was thought to inhibit ferric iron reduction by *Staph. aureus* because nitrate accepts

electrons more readily than ferric iron in this system. Obuekwe (1986) demonstrated that the ferric reductase in *S. putrefaciens* strain 200 was inducible and that ferric iron reduction in intact cells was inhibited by sodium amytal, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO), and sodium cyanide, suggesting involvement of a cytochrome pathway in transferring electrons from donor to ferric iron. He was, however, unable to demonstrate ferric iron reducing activity with cell membranes or with periplasmic or cytoplasmic fractions from the cells. *S. putrefaciens* 200R grown separately on Fe(III), Mn(IV), U(VI), SO_3^{2-}, or $S_2O_3^{2-}$ as terminal electron acceptor under microaerobic or anaerobic conditions was able to reduce Fe(III). This was not the case when this culture was grown separately on O_2, NO_3^-, NO_2^-, or trimethylamine *N*-oxide under otherwise similar conditions (Blakeney et al., 2000). A kinetic study of ferric iron reduction by *S. putrefaciens* strain 200 grown anaerobically on Fe(III) indicated that the form of soluble ferric iron species determines the reaction rate (Arnold et al., 1986b).

The most direct evidence for enzymatically catalyzed ferric iron reduction derives from studies with *Geobacter metallireducens* (strain GS-15), *G. sulfurreducens*, three strains of *Shewanella putrefaciens*, and *Desulfuromonas acetoxidans*. Fe(III) reduction as a form of anaerobic respiration is readily demonstrable with growing and resting cultures of *G. metallireducens*, a strict anaerobe isolated from freshwater sediment (Lovley and Phillips, 1988a), *G. sulfurreducens* isolated from a hydrocarbon-contaminated ditch (Caccavo et al., 1994), and the facultative anaerobe *S.* (formerly *Pseudomonas*, *Alteromonas*) *putrefaciens* strains MR-1, 200, and ATCC 8071 (Myers and Nealson, 1988; Obuekwe et al., 1981; Lovley et al., 1989a). Strain MR-1 was isolated from sediment from Lake Oneida, NY, and strain 200 from a Canadian oil pipeline. All were found to use Fe(III) and Mn(IV) anaerobically as terminal electron acceptor for growth.

In initial lab experiments, *G. metallireducens* was shown to reduce amorphous iron oxide to magnetite (Fe_3O_4), but it did not readily reduce crystalline iron oxides (Lovley and Phillips, 1986b). Roden and Urrutia (1999) showed later that removal of ferrous iron from the surface of crystalline iron oxide was necessary for ready attack by the organism. *G. metallireducens* can use acetate (Fig. 15.9), ethanol, butyrate, and propionate as electron donors. It oxidizes the acetate to CO_2 in the process. In studies with intact cells (Gorby and Lovley, 1991), the organism conveyed reducing power (electrons) from an appropriate electron donor to ferric iron via a membrane-bound electron transport system that included b- but not c-type cytochrome and Fe(III) reductase. The electron transport inhibitors 2-heptyl-4-hydroxyquinoline-*N*-oxide, sodium azide, and sodium cyanide had no effect on the system. *G. metallireducens* oxidizes acetate via the tricarboxylic (citric) acid cycle (see Chap. 6) (Champine and Goodwin, 1991). Proposed sites of energy conservation are electron transfer from NADH to oxidized menaquinone and from reduced cytochrome c_7, a low

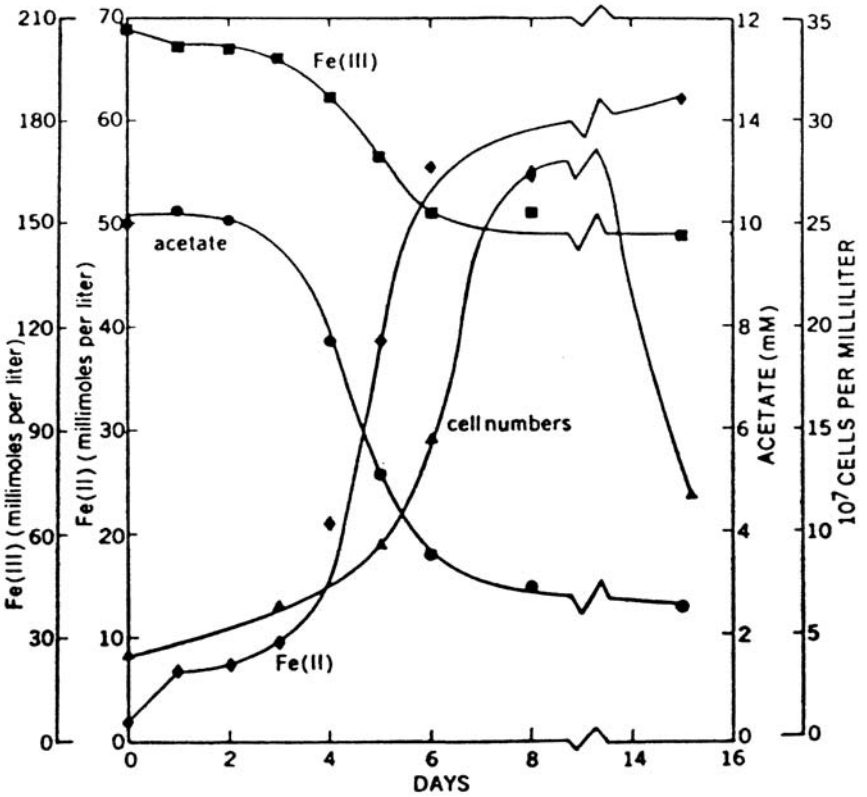


FIG. 15.9 Reduction of ferric iron by acetate through the mediation of anaerobic bacterial strain GS-15 (*Geobacter metallireducens*). This family of curves illustrates the reduction of oxalate-extractable Fe(III) to Fe(II) at the expense of acetate consumption during growth of the culture in FWA medium containing amorphic Fe(III) oxide. (Reproduced from Lovley and Phillips, 1988a, with permission.)

potential cytochrome (≤ -91 mV) to oxidized terminal oxidase (Champine et al., 2000).

G. sulfurreducens, unlike *G. metallireducens*, can use either H_2 or acetate as electron donor in iron(III) reduction. Figure 15.10 shows diagrammatically how the organism uses H_2 in this process. Elemental sulfur, Co(III)-EDTA, fumarate, and malate can serve as alternative electron acceptors, but not Mn(IV) or U(VI). Unlike *G. metallireducens*, the organism cannot use propionate, butyrate, benzoate, or phenol as electron donor, nor can it use some other organic compounds as reductants of Fe(III). The organism cannot reduce nitrate, sulfate, sulfite, or thiosulfate with acetate as electron donor (Caccavo et al., 1994).

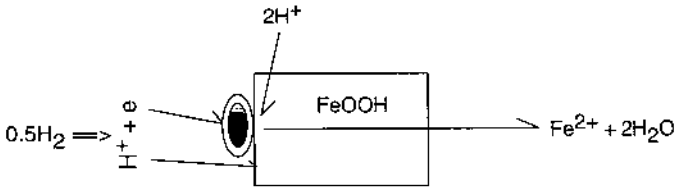


FIG. 15.10 Diagrammatic representation of the reduction of iron(III) in the form of FeOOH with H_2 by an organism such as *Geobacter sulfurreducens* or *Shewanella putrefaciens*. The overall reaction is $0.5H_2 + 2H^+ + FeOOH \rightarrow Fe^{2+} + 2H_2O$.

Shewanella putrefaciens can use H_2 (Fig. 15.10) as well as formate, lactate, and pyruvate as electron donors in the anaerobic reduction of Fe(III), but the last two donors are only incompletely oxidized to acetate and CO_2 (Lovley et al., 1989a; Myers and Nealson, 1988, 1990). Myers and Nealson (1990) found that energy was conserved when *S. putrefaciens* strain MR-1, renamed *S. oneidensis* by Venkateswaran et al. (1999), reduced ferric iron with lactate as electron donor anaerobically. They demonstrated respiration-linked proton translocation, which was completely inhibited by 20 μM carbonylcyanide *m*-chlorophenylhydrazone and partially to completely inhibited by 50 μM 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide.

When grown anaerobically, *S. putrefaciens* has 80% of its cytochrome complement (mostly c-type cytochrome) in the outer membrane of its cell envelope, with the rest (c- and b-type cytochromes) being mostly or entirely associated with the plasma membrane. When grown aerobically, the same organism contains a major portion of its cytochrome complement in its plasma membrane, which according to existing studies, is the more common location of cytochromes in other bacteria, except for periplasmic c-type cytochrome (Myers and Myers, 1992a). Similarly, slightly more than 50% of formate-dependent ferric reductase activity of anaerobically grown *S. putrefaciens* was found associated with the outer membrane, the rest with the plasma membrane. The membranes of aerobically grown cells were devoid of this activity (Myers and Myers, 1993). Most of the formate dehydrogenase activity was soluble (Myers and Myers, 1993), suggesting a possible periplasmic location, as with fumarase reductase in anaerobically grown cells of this organism (Myers and Myers, 1992b). Addition of nitrate, nitrite, fumarate, or trimethylamine *N*-oxide as alternative electron acceptor did not inhibit ferric reductase activity; NADH was able to replace formate as electron donor in experiments with a membrane fraction containing ferric reductase (Myers and Myers, 1993).

Outer membrane cytochromes encoded in genes Omc*A* and Omc*B* in *Shewanella putrefaciens* MR-1 are required for reduction of Mn(IV) oxide, which is insoluble. However, these cytochromes are not involved in the reduction

of soluble electron acceptors, including ferric citrate, nitrate, thiosulfate, and others, not even for FeOOH, which has limited solubility (Myers and Myers, 2001). The studies of Myers and Myers support a model in which, as in the reduction of Mn(IV) oxide, outer membrane proteins are involved in the reduction of insoluble iron oxides anaerobically by *S. putrefaciens*. Lower et al. (2001) obtained direct evidence for the involvement of surface contact between *S. putrefaciens* MR-1 and goethite by atomic force electron microscopy during attack of the goethite under anaerobic conditions. Their results support a model in which the contact enables electron transfer at the cell/mineral interface by means of a 150 kD iron reductase.

In *Geobacter sulfurreducens*, a 300 kDa Fe^{3+}-reductase complex has been detected, of which 67% is located in the outer membrane and the rest in the cytoplasmic membrane (Magnuson et al., 2000). The protein complex includes a 90 kDa c-type cytochrome and flavin adenine dinucleotide (FAD) and has an E_h of -100 mV. It is reduced by NADH but not by H_2. Its Fe(III)-reducing activity is inhibited by rotenone, myxathozole, quinacrine, or *p*-chloromercuribenzoate but not by inhibitors that act on the ubiquinone : cytochrome c oxidoreductase.

Lovley (2000) proposed a model of an Fe^{3+}-reductase system in *G. sulfurreducens* that consists of a 41 kDa cytochrome located in the outer membrane, which conveys electrons to bulk-phase Fe(III) at the exterior of the outer membrane. This cytochrome receives electrons from NADH in a sequence of redox reactions involving an NADH-dehydrogenase complex and a 89 kDa cytochrome in the plasma membrane, and a 9 kDa cytochrome in the periplasm. This model has yet to be integrated with the findings of Magnuson et al. (2000). An alternative model has been proposed in which an extracellular 9.6 kDa c-type cytochrome released by *G. sulfurreducens* acts as an electron shuttle between the cell and bulk-phase Fe(III). This model is based on finding such a cytochrome in spent medium from *G. sulfurreducens* cultures (Seeliger et al., 1998). Strong evidence against this model makes it unlikely, however (Lloyd et al., 1999).

As previously mentioned, *S. putrefaciens* strain 200 forms a constitutive and an inducible Fe(III) reductase. The latter is induced only anaerobically. Both reductases are responsible for rapid Fe(III) reduction under anaerobic conditions. The constitutive reductase will reduce Fe(III) at low O_2 tension, but the rate of Fe(III) reduction is very slow. Apparently, competition with O_2 is responsible for this slow rate of reduction. A branched respiratory pathway is postulated in which one branch leads to O_2 and the other to Fe(III) (Arnold et al., 1990).

Not all Fe(III) reductases function in dissimilatory iron reduction. The iron reductase activity detected in *Pseudomonas aeruginosa* (Cox, 1980) evidently represents an assimilatory iron reductase system because it reduced only complexed iron such as ferripyrochelin and ferric citrate (pyrochelin and citrate are known siderophores). The ferric reductase had a cytoplasmic location,

whereas the ferripyrochelin reductase was located in the periplasm and cytoplasm. The activities appear to be linked to pyridine nucleotide although reduced glutathione was also able to serve as electron donor. Similarly, the iron(III) reduction observed with *Escherichia coli* K12 (Williams and Poole, 1987) involved an assimilatory iron reductase. Like *P. aeruginosa*, *E. coli* K12 reduced ferric citrate directly with reduced pyridine nucleotide as electron donor without involvement of the respiratory chain in the membrane of the organism. Adenosine 5′-triphosphate (ATP) and cyanide were found to stimulate ferric citrate reduction, possibly by forming complexes with Fe(III).

Bioenergetics of Dissimilatory Iron Reduction

Because proton translocation has been demonstrated during Fe(III) reduction by *S. putrefaciens* MR-1 (Myers and Nealson, 1990), it can be inferred that this organism conserves energy chemiosmotically in this process. The other strains of *S. putrefaciens*, such as those studied by Obuekwe (1986), Arnold et al. (1986a, 1986b) and Lovley et al. (1989a), probably conserve energy by the same mechanism as strain MR-1. At least they grow anaerobically when Fe(III) is the only terminal acceptor but not in its absence or in the absence of an alternative acceptor. *Geobacter metallireducens* most likely conserves energy chemiosmotically in Fe(III) respiration because, as previously mentioned, electrons from a suitable energy source are transported to Fe(III) by a membrane-bound system that includes b-type cytochrome and an Fe(III) reductase (Gorby and Lovley, 1991).

Free energy calculations show that when acetate is the electron donor (reductant) and Fe^{3+} is the electron acceptor (oxidant), the standard free energy change at pH 7 ($\Delta G_r^{\circ\prime}$) is $-193.4\,\text{kcal mol}^{-1}$ ($-808.4\,\text{kJ mol}^{-1}$). This value is close to that when O_2 is the electron acceptor ($-201.8\,\text{kcal mol}^{-1}$ or $-843.5\,\text{kJ mol}^{-1}$). However, when $Fe(OH)_3$ is the oxidant of acetate, the standard free energy change at pH 7 is only $-5.5\,\text{kcal mol}^{-1}$ ($-23.0\,\text{kJ mol}^{-1}$) (Ehrlich, 1993). This indicates that undissolved iron oxides or hydroxides are poor electron acceptors at neutral or alkaline pH and do not allow for the magnitude of biochemical energy conservation that dissolved Fe(III) does. The iron oxides are better acceptors and more effective for biochemical energy conservation somewhat below pH 7.0. Nevertheless, conditions exist in nature in which minerals containing ferric iron serve effectively as electron acceptors without the ferric iron first being dissolved.

Ferric Iron Reduction as an Electron Sink

As previously mentioned, it has been suggested that not all iron(III)-reducing bacteria gain energy in the process. Ghiorse (1988) and Lovley (1991) proposed

that a number of bacteria reduce ferric iron merely to dispose of excess reducing power via secondary respiratory pathways without conserving energy or that the iron reduction that was observed with these organisms was part of their iron assimilation process. These interpretations ignore the possibility that an absence of growth stimulation under anaerobic conditions when an organism reduces Fe(III) with stoichiometric release of extracellular Fe^{2+} is not necessarily an indication that energy is not conserved in the reduction. The Fe(III) reduction process may simply yield insufficient energy for growth if it is the only source of energy and may have to be accompanied by an additional respiratory or fermentative process to meet the full energy demand. In other words, Fe(III) reduction is an ancillary source of energy for these particular organisms.

The observation by Lovley et al. (1995) that *Pelobacter carbinolicus* can catabolize 2,3-butanediol with the production of ethanol and acetate by fermentation as well as by reduction of Fe(III) or S^0 may provide new insight into this puzzle. The authors noted that the ratio of ethanol to acetate produced from 2,3-butanediol was significantly greater in the absence of Fe(III) than in its presence. Because *P. carbinolicus* lacks c-type cytochromes, it may be using the Fe(III) merely as an electron sink in this case. On the other hand, the authors also found that the organisms can grow on H_2 as sole energy source in a medium in which acetate is the sole carbon source (it cannot use acetate as an energy source) and Fe(III) is the only terminal electron acceptor. Under these conditions the organism must respire and conserve energy chemiosmotically. This raises the question of whether when growing on 2,3-butylenediol, the organism uses Fe(III), when present, merely as an electron sink and does not conserve energy from its reduction.

Reduction of Ferric Iron by Fungi

Some fungi seem to be able to reduce ferric iron. Lieske reported such a phenomenon (see Starkey and Halvorson, 1927). It was also observed by Ottow and von Klopotek (1969). They noted reduction of ferric iron in hematite (Fe_2O_3) by *Alternaria tenuis*, *Fusarium oxysporum*, and *F. solani*, all of which possessed an inducible nitrate reductase. Fungi tested in this study that were incapable of reducing nitrate were also incapable of reducing ferric iron. Ottow and von Klopotek concluded from their findings that nitrate reductase in fungi that possess it functions in the reduction of ferric iron as well as nitrate, a mechanism thus similar to that of many bacteria. Their conclusion, however, raises a question as to where in the fungi the nitrate reductase is located to be able to act on hematite and whether this represents dissimilatory or assimilatory iron reduction. The latter seems more likely. In the simultaneous presence of nitrate and hematite, it is possible that nitrite produced from the nitrate reduces Fe(III) in

hematite nonenzymatically, as in the case of *S. putrefaciens* (Obuekwe et al., 1981).

Types of Ferric Compounds Attacked by Dissimilatory Iron(III) Reduction

The ease with which bacteria reduce ferric iron depends in part on the form in which they encounter it. In the case of insoluble forms, the order of decreasing solubilization in one study (Ottow, 1969b) was $FePO_4 \cdot 4H_2O > Fe(OH)_3 >$ lepidochrosite (γ-FeOOH) > goethite (α-FeOOH) > hematite (α-Fe_2O_3) when *Bacillus polymyxa*, *B. sphaericus*, and *Pseudomonas aeruginosa* were used with glucose as electron donor. The order was inverted with respect to $FePO_4 \cdot 4H_2O$ and $Fe(OH)_3$ when *Enterobacter* (*Aerobacter*) *aerogenes* and *B. mesentericus* were tested. In another study (De Castro and Ehrlich, 1970), using glucose as electron donor, marine *Bacillus* 29A was found to solubilize larger amounts of iron from limonite and goethite than from hematite. In initial stages of the reduction, the order of decreasing activity was goethite > limonite > hematite. The bacterial iron-solubizing activity in these experiments was enhanced by the addition of phenosafranin.

Bacillus 29, the wild-type parent of strain 29A, did not reduce ferric iron extensively when it occurred in ferromanganese nodules from the deep sea even though it reduced the Mn(IV) oxide (Ehrlich et al., 1973). Because of the insoluble nature of the iron(III) in the nodules, the aerobic conditions, and in some cases the circumneutral pH at which these observations were made, it seems unlikely that the absence of measurable iron reduction was due to the manganese inhibition phenomenon described by Lovley and Phillips (1988b). More likely it was related to the significantly greater amount of free energy available from Mn(IV) oxide reduction than from ferric oxide reduction (see Ehrlich, 1993). In a study of anaerobic reduction of pedogenic iron oxides by *Clostridium butyricum*, Munch and Ottow (1980) found that amorphous oxides were more readily attacked than crystalline oxides. This was also observed by Lovley and Phillips (1986b) in anaerobic experiments with an enrichment culture from Potomac River sediment tested on various forms of Fe(III).

In a study with *Acidiphilium* SJH, Bridge and Johnson (2000) found that the order of decreasing Fe(III) reduction was dissolved Fe^{3+} > amorphous $Fe(OH)_3$ > magnetite > goethite = natrojarosite > akaganeite > jarosite > hematite (no significant dissolution). In this case, the mineral was not attacked directly by the organism. The authors found that the bacterial reduction of the iron in the minerals depended on the abiotic mobilization of Fe(III) by the acidity of the medium, which was pH 2.0 initially. The abiotic Fe(III) mobilization was

enhanced by bacterial reduction of the solubilized Fe(III), which resulted in product removal in the abiotic dissolution step.

Although magnetite had been thought to be resistant to microbial reductive attack, its anaerobic reductive dissolution to Fe^{2+} by two strains of *Shewanella putrefaciens* using glucose and lactate as electron donors has now been observed (Kostka and Nealson, 1995). Although not mentioned by Hilgenfeldt (2000), the extensive dissolution of biogenic magnetite he observed in the surface sediments of the Benguela upwelling system may have been due to the activity of Fe(III)-reducing bacteria.

The anaerobic reduction by *S. putrefaciens* strain MR-1 and by some unidentified bacteria of ferric iron that replaces some of the Al in the octahedral sheets of the clay smectite using lactate and formate as electron donors was observed by Stucki et al. (1987) and by Kostka et al. (1996). This microbial action has also been shown with three strains of *Pseudomonas fluorescens* and a strain of *Ps. putida* (Ernstsen et al., 1998). Interestingly, the reduced iron (Fe^{2+}) in the clay is not mobilized in this reduction. Stucki and Roth (1977) proposed that the reduction proceeds in two steps. In the first step, an increase in layer charge without structural change occurs during initial reduction of some Fe(III), and in the second step, constant layer charge is maintained as a result of elimination of structural OH accompanied by a decrease of the iron in the octahedral sheet during subsequent reduction of additional Fe(III). The reduction of Fe^{3+} in the octahedral sheet of smectite results in an increase in net surface charge of the mineral (Stucki et al., 1984) and a decrease in its swellability (Stucki et al., 2000). The mechanism whereby the bacteria inject electrons into the smectite to reduce the structural iron remains to be elucidated.

Because oxides of ferric iron are very insoluble at near-neutral pH, the question arises as to how bacteria can attack these compounds. The tendency of the oxides to dissociate into soluble species in water at near-neutral pH is simply too small to explain the phenomenon. Physical contact between the active cell and the surface of the oxide particle appears essential. This was first demonstrated by Munch and Ottow (1982) in experiments in which they placed iron oxide inside a dialysis bag whose pores did not permit the passage of bacterial cells into the bag but did permit ready diffusion of culture medium with its dissolved inorganic and organic chemical species. No iron reduction occurred when bacteria (*Clostridium butyricum* or *Bacillus polymyxa*) were placed in medium outside the bag, but it did occur when bacteria and iron oxide were both present inside the bag. Interestingly, metabolites, including acids, produced by the bacteria were not able to dissolve significant amounts of oxide. Furthermore, the lowered E_h (redox potential) generated by the bacteria, whether inside or outside the dialysis bag, was also unable to cause reduction of ferric iron in the oxide. Nevin and Lovley (2000) concluded that some bacteria mobilize ferric iron in oxide minerals as ferrous iron by direct attack. The model for the Fe(III) reductase system in

Geobacter sulfurreducens (see Lovley, 2000), previously discussed, illustrates how direct attack by bacteria at the surface of a ferric oxide particle could occur.

15.7 NONENZYMATIC OXIDATION OF FERROUS IRON AND REDUCTION OF FERRIC IRON BY MICROBES

Nonenzymatic Oxidation

Many different kinds of microorganisms can promote the oxidation of ferrous iron indirectly (i.e., nonenzymatically). They accomplish this by affecting the redox potential of the environment. This means they release into their surroundings metabolically formed oxidants that have the ability to oxidize ferrous iron. They can also accomplish this by generating a pH above 5 at which Fe^{2+} is oxidized by oxygen in the air (*autoxidation*). Among the first to recognize indirect iron oxidation by microbes were Harder (1919), Winogradsky (1922), and Cholodny (1926). Starkey and Halvorson (1927) demonstrated indirect oxidation of iron by bacteria in laboratory experiments. They explained their findings in terms of reaction kinetics involving the oxidation of ferrous iron and the solubilization of ferric iron by acid. From their work it can be inferred that any organism that raises the pH of a medium by forming ammonia from protein-derived material [Eq. (15.25)] or by consuming salts of organic acids [Eq. (15.26)] can promote ferrous iron oxidation in an aerated medium:

$$\underset{\substack{|\\ NH_2\\ \text{amino acid}}}{RCHCOOH} + H_2O + 0.5O_2 \rightarrow \underset{\substack{\|\\ O\\ \text{keto acid}}}{RCCOOH} + NH_4^+ + OH^- \qquad (15.25)$$

$$\underset{\text{lactate}}{C_3H_5O_3^-} + 3O_2 \rightarrow 3CO_2 + 2H_2O + OH^- \qquad (15.26)$$

A more specialized case of indirect microbial iron oxidation is that associated with cyanobacterial and algal photosynthesis. The photosynthetic process may promote ferrous iron oxidation by creating conditions that favor autoxidation in two ways: (1) by raising the pH of the waters in which they grow (bulk phase) and (2) by raising the oxygen level in the waters around them. The pH rise is explained by the equations:

$$2HCO_3^- \rightarrow CO_3^{2-} + CO_2(g) + H_2O \qquad (15.27)$$

$$CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^- \qquad (15.28)$$

Reaction (15.27) is promoted by CO_2 assimilation in oxygenic photosynthesis, the reaction of which may be summarized as:

$$CO_2 + H_2O \xrightarrow[\text{light}]{\text{Chl}} CH_2O + O_2 \tag{15.29}$$

Reaction (15.29) also yields much of the oxygen needed for autoxidation of the iron. Its genesis causes a rise of E_h because of increased oxygen saturation or even supersaturation of the water around the cyanobacterial or algal cells.

Ferrous iron may be protected from chemical oxidation at elevated pH and E_h by chelation with gluconate, lactate, or oxalate, for example. In that instance, bacterial breakdown of the ligand will free the ferrous iron, which then autoxidizes to ferric iron. This has been demonstrated in the laboratory with a *Pseudomonas* and a *Bacillus* strain (Kullman and Schweisfurth, 1978). These cultures do not derive any energy from iron oxidation but rather from the oxidation of the ligand.

The production of ferric iron from the oxidation of ferrous iron at pH values above 5 usually leads to precipitation of the iron. But the presence of chelating agents such as humic substances, citrate, and others can prevent the precipitation. Unchelated ferric iron tends to hydrolyze at higher pH values and may form compounds such as ferric hydroxide:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \tag{15.30}$$

Ferric hydroxide is relatively insoluble and will settle out of suspension. It may crystallize and dehydrate, forming FeOOH, goethite ($Fe_2O_3 \cdot H_2O$), or hematite (Fe_2O_3). In excess base, $Fe(OH)_3$ may, however, form water-soluble, charged entities such as $Fe(OH)_2O^-$ or $Fe(OH)O_2{}^{2-}$ because $Fe(OH)_3$ has amphoteric properties.

Nonenzymatic Reduction

Starkey and Halvorson (1927) tried to explain ferric iron reduction in nature as an indirect effect of microbes. They argued that with a drop in pH and lowering in oxygen tension caused by microbes, ferric iron would be changed to ferrous iron according to the relationship

$$4Fe^{2+} + O_2 + 10H_2O \Leftrightarrow 4Fe(OH)_3 + 8H^+ \tag{15.31}$$

in which Fe(III) was considered to be an insoluble phase [$Fe(OH)_3$]. From this chemical equation they derived the relationship

$$[Fe^{2+}] = K\frac{[H^+]^2}{[O_2]^{1/4}} \tag{15.32}$$

But as the experiments of Munch and Ottow (1982) convincingly showed, this mode of iron reduction is usually not very significant in nature. This was also shown by Lovley et al. (1991). Iron oxide minerals are relatively stable in the absence of oxygen when a strong reducing agent is not present. In the presence of such reducing agents, however, chemical Fe(III) reduction does occur. For instance, H_2S produced by sulfate-reducing bacteria may reduce ferric to ferrous iron before precipitating ferrous sulfide (Berner, 1962).

$$2FeOOH + 3H_2S \xrightarrow{\text{pH } 7-9} 2FeS + S^0 + 4H_2O \quad (15.33)$$

$$2FeOOH + 3H_2S \xrightarrow{\text{pH } 4} FeS + FeS_2 + 4H_2O \quad (15.34)$$

Marine bacteria that disproportionate elemental sulfur into H_2S and sulfate have been shown to chemically reduce Fe(III) and Mn(IV) anaerobically (Thamdrup et al., 1993):

$$4S^0 + 4H_2O \rightarrow 3H_2S + {SO_4}^{2-} + 2H^+ \quad (15.35)$$

Formate produced by a number of bacteria (e.g., *Escherichia coli*) can reduce Fe(III):

$$2Fe^{3+} + HCOOH \rightarrow 2Fe^{2+} + 2H^+ + CO_2 \quad (15.36)$$

Some other metabolic products can also act as reductant of ferric iron (see discussion by Ghiorse, 1988). In general, reduction is favored by acid pH.

With the discovery that some Fe(III)-reducing organisms can reduce humic substances (Lovley et al., 1996, 1998; Scott et al., 1998), it has been proposed that the microbially reduced humic substances may reduce Fe(III) in soil abiologically, with the hydroquinone groups of the humics acting as electron shuttle between the cells and extracellular Fe(III) (Lovley, 2000).

15.8 MICROBIAL PRECIPITATION OF IRON

Enzymatic Processes

The clearest example of enzymatic iron(III) precipitation is that of *Gallionella ferruginea*. The ferric iron (FeOOH) it produces in its oxidation of ferrous iron is deposited in its stalk fibrils by a mechanism that is still unclear at this time (see discussion by Ghiorse, 1984; Ghiorse and Ehrlich, 1992). Enzymatic iron reduction by other organisms can also result in precipitation of magnetite (Fe_3O_4) (Lovley et al., 1987) and in precipitation of Fe(II) as siderite ($FeCO_3$) (Coleman et al., 1993) (see Chap. 8).

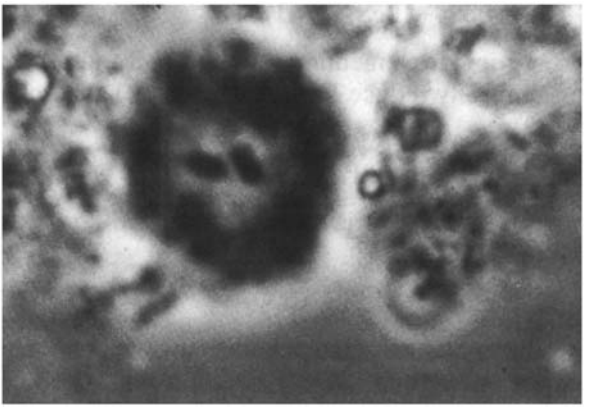


FIG. 15.11 *Siderocapsa geminata*, Skuja (1956) (×7000). Specimen from filtered water from Pluss See, Schleswig-Holstein, Germany. Note the capsule surrounding the pair of bacterial cells. (Courtesy of W. C. Ghiorse and W.-D. Schmidt.)

Nonenzymatic Processes

Some bacteria may deposit ferric oxides on their cells that they formed nonenzymatically. This nonenzymatic oxidation may involve ligand destruction of iron chelates (e.g., Aristovskaya and Zavarzin, 1971). Such organisms include sheathed bacteria such as *Leptothrix* spp. (Fig. 15.7), *Siderocapsa* (Fig. 15.11), *Naumanniella*, *Ochrobium*, *Siderococcus*, *Pedomicrobium*, *Herpetosyphon*, *Seliberia* (Fig. 15.12), *Toxothrix* (Krul et al., 1970), *Acinetobacter* (MacRae and Celo, 1975), and *Archangium*. The precipitation on the cell probably involves acid exopolymer (glycocalyx) in most if not all instances (see next section).

Bioaccumulation of Iron

Most or all of the bacteria listed in the previous section may also accumulate ferric iron produced either enzymatically or nonenzymatically by other organisms in the bulk phase. Usually the iron is passively collected on the cell surface by reacting with acidic exopolymer, which exposes negative charges. The exopolymer may be organized in the form of a sheath, a capsule, or slime (see discussion by Ghiorse, 1984). Some protozoans such as *Anthophysa*, *Euglena* (Mann et al., 1987), *Bikosoeca*, and *Siphomonas* are also known to deposit iron on their cells.

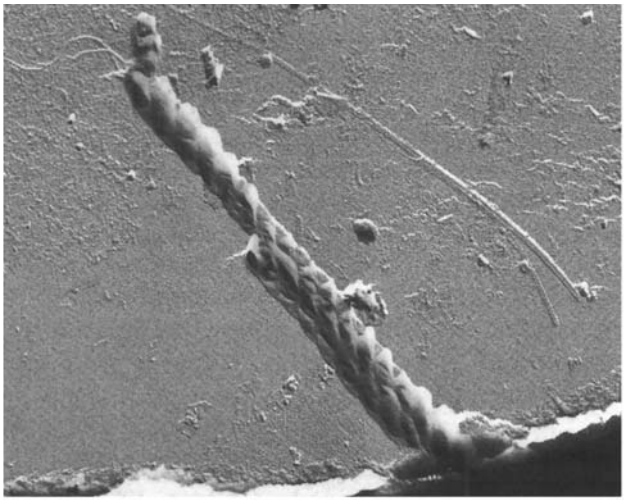


FIG. 15.12 *Seliberia* sp. from forest pond neuston (×8200). (Courtesy of W. C. Ghiorse.)

15.9 THE CONCEPT OF IRON BACTERIA

A survey of the literature on bacteria that interact with iron shows that the term *iron bacteria* is differently defined by different authors. Some have included in this term any bacteria that precipitate iron, whether by active (enzymatic) oxidation or by passive accumulation of ferric oxides or hydroxides about their cells, whether they possess cellular structures with specific affinity for ferric iron or not. As Starkey (1945) suggested, the term "iron bacteria" is best reserved for those bacteria that oxidize iron enzymatically. Bacteria that passively accumulate iron should be called *iron accumulators*, among which *specific* and *nonspecific* accumulators should be recognized as subgroups.

Magnetotactic bacteria occupy a special position among iron bacteria (Blakemore, 1982) (Fig. 15.13). They are motile organisms that have the capacity to take up complexed ferric iron and transform it into magnetite (Fe_3O_4) by a mechanism that seems to involve reduction and partial reoxidation (Frankel et al., 1983; Bazylinski and Frankel, 2000). The magnetite is deposited as crystals in membrane-bound structures called *magnetosomes* (Fig. 15.13) (Blakemore, 1982). The magnetite crystals are single-domain magnets that cause the bacteria that form them to align with the Earth's magnetic field, which is inclined downward in the northern hemisphere and upward in the southern hemisphere. This helps the bacteria in locating their preferred habitat, a partially reduced

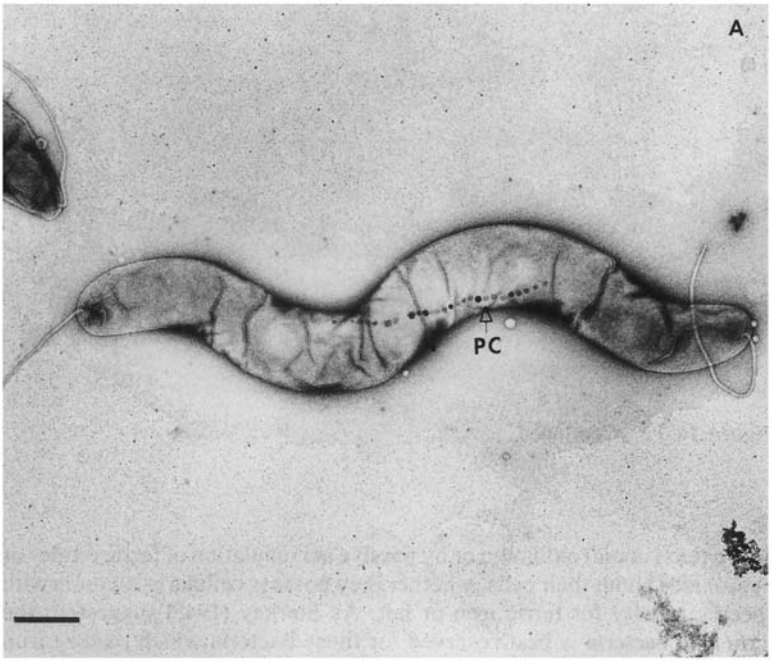


FIG. 15.13 *Aquaspirillum magnetotacticum*. (A) Transmission electron micrograph of a negatively stained cell, showing an electron-dense particle chain (PC) of magnetosomes (bar = 500 nm). (Reproduced from Balkwill et al., 1980, with permission).

environment. Because, by convention, the magnetic field direction is defined in terms of the north-seeking end of a compass needle, magnetic polarity in magnetotactic bacteria in the northern hemisphere influences them to move downward. Of necessity, opposite polarity in magnetotactic bacteria in the southern hemisphere helps them to locate their preferred habitat (Blakemore, 1982). Upon the death of magnetotactic bacteria, the magnetite crystals in them are liberated and in nature may become incorporated in sediment. It has been suggested that remanent magnetism detected in some rocks may be due to magnetite residues from magnetotactic bacteria (Blakemore, 1982; Karlin et al., 1987). However, magnetite is also formed extracellularly by some nonmagnetotactic bacteria (Lovley et al., 1987) (see Sec. 15.10).

15.10 SEDIMENTARY IRON DEPOSITS OF PUTATIVE BIOGENIC ORIGIN

Sedimentary iron deposits, many representing large ore bodies, may feature iron in the form of oxides, sulfides, or carbonates. Microbes appear to have

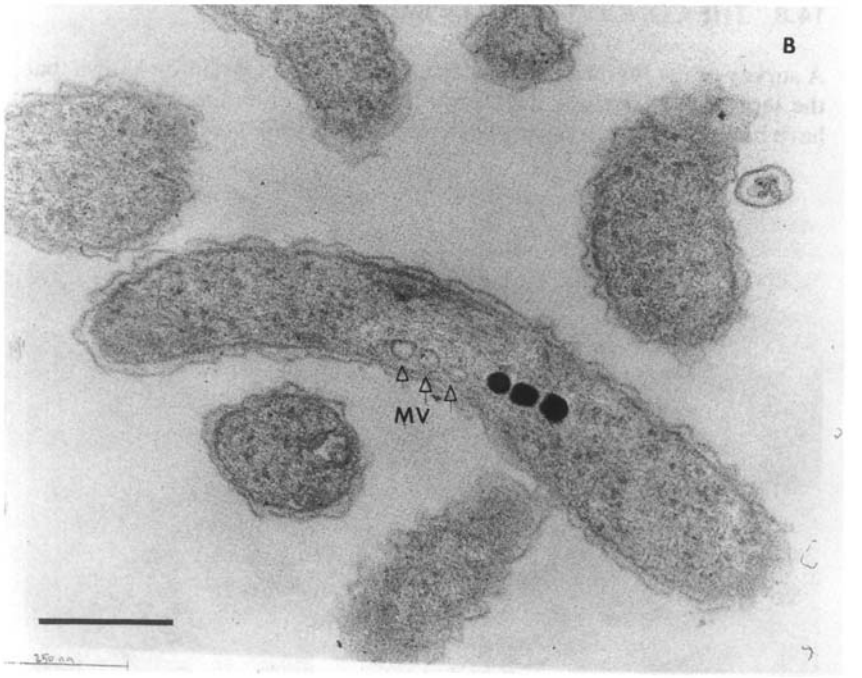


FIG. 15.13 *Aquaspirillum magnetotacticum*. (B) Transmission electron micrograph of thin sections showing trilaminate structure of the membranous vesicles (MV), which lie along the same axis as complete magnetosomes (bar = 250 nm). (Reproduced from Gorby et al., 1988, with permission.)

participated in the formation of many of these deposits (Konhauser, 1997). Their participation can be inferred from (1) the presence in some deposits of fossilized microbes with imputed iron-oxidizing or -accumulating potential; (2) the presence of living iron-oxidizing or iron-accumulating bacteria in currently forming deposits of ferric oxide (Fe_2O_3 or FeOOH); (3) the presence of Fe(III)-reducing bacteria in currently forming deposits of magnetite (Fe_3O_4); or (4) the presence of sulfate-reducing bacteria in currently forming iron sulfide deposits. The probable environmental conditions prevailing at the time of formation may be inferred from an iron deposit of proven biogenic origin. Thus, identification of microfossils in an iron deposit may permit the deduction of prevailing environmental conditions from knowledge of environmental requirements and activities of modern microorganisms that resemble the microfossils. In this section only iron oxide deposits are considered. Biogenic iron sulfide formation will be discussed in Chapter 19. Biogenic iron carbonate (siderite) formation was briefly treated in Chapter 8.

Among the most ancient iron deposits in the formation of which microbes may have played a central role are the Banded Iron Formations (BIF) that arose mostly in the Precambrian, in the time interval roughly between 3.3 and 1.8 eons ago. The major deposits were formed between 2.2 and 1.9 eons ago (see discussions by Cloud, 1973; Lundgren and Dean, 1979; Walker et al., 1983; Nealson and Myers, 1990). These formations have been found in various parts of the world and in many places are extensive enough to be economically exploitable as iron ore. They are characterized by alternating layers rich in chert, a form of silica (SiO_2), and layers rich in iron minerals such as hematite (Fe_2O_3), magnetite (Fe_3O_4), the iron silicate chamosite, and even siderite ($FeCO_3$). Ferric iron predominates over ferrous iron in the iron-rich layers. Average thickness of the layers is 1–2 cm, but they may be thinner or thicker. Because the most important BIF were formed during that part of the Precambrian when the atmosphere around the Earth changed from reducing or nonoxidizing to oxidizing due to the emergence and incremental dominance of oxygenic photosynthesis, Cloud (1973) argued that the alternating layers in BIF reflected episodic deposition of iron oxides, involving seasonal, annual, or longer period cycles. In the reducing or nonoxidizing atmosphere of the Archean, iron in the Earth's crust was mostly in the ferrous form and thus could act as a scavenger of oxygen initially produced by oxygenic photosynthesis. The scavenging reaction involved autoxidation of the iron. Because autoxidation of iron is a very rapid reaction at near neutral pH and higher, the supply of ferrous iron could have been periodically depleted. Further oxygen scavenging would then have had to wait until the supply of ferrous iron was replenished by leaching from rock or hydrothermal emissions (Holm, 1987). Alternatively, if much of the ferrous iron for scavenging O_2 was formed by ferric iron–respiring bacteria while they consumed the organic carbon produced by seasonally growing photosynthesizers, periodic depletion of organic carbon rather than ferrous iron could have been the basis for the iron-poor layers (Nealson and Myers, 1990). Bacterial reduction of oxidized iron could also explain the origin of magnetite-rich layers of BIF; Lovley et al. (1987) and Lovley and Phillips (1988a) found in their laboratory experiments that *Geobacter metallireducens* precipitated magnetite in reducing Fe^{3+}. Previously it had been proposed that the magnetite in the iron-rich layers may have resulted from partial reduction of hematitic iron by organic carbon from biological activity (Perry et al., 1973).

An ongoing process of iron deposit development in a hot spring may provide a model for the origin of some BIF (Pierson and Parenteau, 2000). It involves microbial mats consisting of cyanobacteria. Pierson and Parenteau observed three kinds of mats in Chocolate Pots Hot Springs in Yellowstone National Park. One kind consisted of *Synechocystis* and *Chloroflexus* and another of *Pseudoanabaena* and *Mastigocladus*. Both of these were present at 48–54°C. A third kind consisted mostly of *Oscillatoria* and occurred at 36–45°C. The iron

minerals occurred below the upper 0.5 mm of the mats, having become entrapped in the biofilm formed by the cyanobacteria. Gliding motility seemed to facilitate the encrustation process of the cyanobacteria. The iron in the water bathing the mats was mostly ferrous. Photosynthetic activity of the mats appears to promote its oxidation (Pierson et al., 1999), leading to the formation of amorphous iron oxides that are the source of the iron deposit. The ultimate development of the mineral deposit involves the death and decay of iron encrusted cells.

If iron was indeed the scavenger of the oxygen of early oxygenic photosynthesis, it would mean that the sediments became increasingly more oxidizing relative to the atmosphere due to the accumulation of the oxidized iron (Walker, 1987). Only when free oxygen began to accumulate would the atmosphere have become oxidizing relative to the sediment. Chert was deposited in BIF because no silicate-depositing microorganisms (e.g., diatoms, radiolarians) had yet evolved. As more oxygen was generated by the photosynthesizers, ferrous iron reserves became largely depleted, permitting the oxygen content of the atmosphere to increase to levels we associate with an oxidizing atmosphere. This in turn would have restricted Fe(III) reduction to the remaining and ever-shrinking oxygen-depleted (anaerobic) environments. Although an extensive array of microfossils has been found in the cherty layers of various BIF (Fig. 15.14) (e.g., Gruner 1923, Cloud and Licari, 1968), not all sedimentologists agree that the origin of BIF depended on biological activity (see discussion by Walker et al., 1983).

Some microbiologists, who have found that some purple photosynthetic bacteria can oxidize Fe(II) anaerobically by using it in place of reduced sulfur in the reduction of CO_2, have suggested that BIF formation may have been initiated before oxygenic photosynthesis had evolved (Widdel et al., 1993). Anbar and Holland (1992) previously suggested that BIF may have begun to form in anoxic Precambrian oceans as a result of abiotic photochemical oxidation of Mn^{2+} to a manganese(IV) oxide like birnessite, which then served as oxidant in the oxidation of ferrous hydroxide complexes to γ-FeOOH or amorphous ferric hydroxide.

Among modern iron deposits in whose formation microorganisms are or have been participating are ochre deposits, bog and similar iron ores, and others. Formation of ochre deposits consisting of amorphous iron oxides (Ivarson and Sojak, 1978) is a common observation in field drains. *Gallionella*, sometimes associated with *Leptothrix*, is usually the causal organism. Hanert (1974b) measured rates of ochre deposition in field drains in terms of ferric iron deposition on submerged microscope slides. He found 4.0, 8.8, and 20.2 µg of iron deposited per square centimeter in 1, 2, and 3 days, respectively. Biogenic ochre deposits were also reported to be forming in a bay (the caldera of Santorini) of the Greek island Palaea Kameni in the Aegean Sea (Puchelt et al., 1973; Holm, 1987). *Gallionella ferruginea* has been identified as the source of this ochre. The

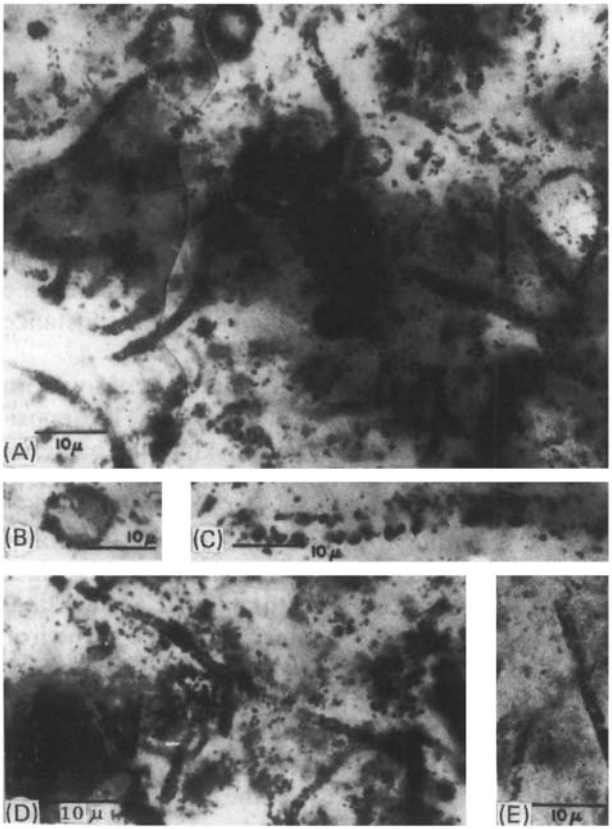


FIG. 15.14 Gunflint microbiota in the stromatolites of the Biwabik Iron Formation, Corsica Mine, Minnesota. (A) *Gunflintia* filaments and *Huroniospora spheroides* replaced by hematite are abundant in some of the dark laminations in stromatolitic rocks at the Corsica Mine. (B) *Huroniospora*, Corsica Mine. (C) Wide filament, Corsica Mine. (D, E) *Gunflintia* filaments, Corsica Mine. (Reproduced from Cloud and Licari, 1968, with permission.)

ferrous iron that is oxidized by *Gallionella* in this instance is of hydrothermal origin.

Bog iron deposition has been observable in the Pine Barrens of southern New Jersey (Crerar et al., 1979; Madsen et al., 1986). The depositional process seems to depend on several kinds of bacteria: *Thiobacillus ferrooxidans*, *Leptothrix ochracea*, *Crenothrix polyspora*, *Siderocapsa geminata*, and an iron-oxidizing *Metallogenium* (Walsh and Mitchell, 1972). According to Crerar et al. (1979), the iron is oxidized and precipitated by the iron-oxidizing bacteria from

acid surface waters, which exhibit a pH range of 4.3–4.5 in summer. It accumulates as limonite (FeOOH)-impregnated sands and silts. The source of the iron is glauconite and to a much lesser extent pyrite in underlying sedimentary formations, from which it is released into groundwater—whether with microbial help has not yet been ascertained. The iron is brought to the surface by groundwater base flow, which feeds local streams. Biocatalysis of iron oxidation seems essential to account for the rapid rate of iron oxidation in the acid waters. However, *T. ferrooxidans* is probably least important because it is only infrequently encountered, perhaps because the environmental pH is above its optimum. Dissolved ferric iron in the streams in the Pine Barrens can be photoreduced (Madsen et al., 1986).

Trafford et al. (1973) describe an example in which *T. ferrooxidans* appeared responsible for ochre formation in field drains from pyritic soils. It is surprising that no jarosite was reported to be formed under these conditions (Silver et al., 1986).

15.11 MICROBIAL MOBILIZATION OF IRON FROM MINERALS IN ORE, SOIL, AND SEDIMENTS

Bacteria and fungi are able to mobilize significant quantities of iron from minerals in ore, soil, and sediment in which the iron is a major constituent. The minerals include carbonates, oxides, and sulfides. Attack of iron sulfide will be discussed in Chapter 19; attack of carbonates was briefly discussed in Chapter 8. In this section the stress is on iron oxides.

As indicated in Section 15.5, iron reduction has been observed for some time with bacteria from soil and aquatic sources. In recent years a more systematic study of the importance of this activity in soils and sediments has been undertaken (see, for instance, review of activity in marine sediments by Burdige, 1993). Ferric iron reduction (iron respiration) can be an important form of anaerobic respiration in environments in which nitrate or sulfate is present in insufficient quantities as terminal electron acceptor and in which methanogenesis is not occurring (Lovley, 1987, 1991, 2000; Sørensen and Jørgensen, 1987; Lowe et al., 2000). Under these conditions it can make an important contribution to anaerobic mineralization of organic carbon. Indeed, the presence of bioreducible ferric iron can inhibit sulfate reduction and methanogenesis when electron donors like acetate or hydrogen are limiting (Lovley and Phillips, 1986a, 1987). Caccavo et al. (1992) found a facultative anaerobe, *Shewanella alga* strain BrY, that oxidized hydrogen anaerobically using Fe(III) as electron acceptor in the Great Bay Estuary, New Hampshire. Evidence that some ferric iron–reducing bacteria can use hydrogen as electron donor was first reported by Balashova and Zavarzin (1979). The form in which ferric iron occurs can determine whether bacterial iron

respiration will occur. In sediments from a freshwater site in the Potomac River estuary in Maryland, only amorphous FeOOH present in the upper 4 cm was bacterially reducible. Mixed Fe(II)-Fe(III) compounds that were present in deeper layers were not attacked (Lovley and Phillips, 1986b). Local E_h and pH conditions can determine the form in which the iron produced in bioreduction of FeOOH will occur. A mixed culture from sediment of Contrary Creek in central Virginia produced magnetite (Fe_3O_4) in the laboratory when the culture medium was allowed to go alkaline (pH 8.5 in the absence of glucose), but it produced Fe^{2+} when the medium was allowed to go acid (to pH 5.5 in the presence of glucose) (Bell et al., 1987). E_h values dropped from a range between 0 and -100 mV to less than -200 mV at the end of the experiments. A growing pure culture of a strict anaerobe, *Geobacter metallireducens* strain GS-15, produced magnetite from amorphous iron oxide directly with acetate as reductant at pH 6.7–7.0 and 30–35°C (Lovley and Phillips, 1988a; Lovley et al., 1987) (see also Sect. 15.5).

The phenomenon of **gleying** in soil has come to be associated with bacterial reduction of iron oxides. It is a process that occurs under anaerobic conditions that may result from waterlogging. The affected soil becomes sticky and takes on a gray or light greenish-blue coloration (Alexander, 1977, p. 377). Although once attributed to microbial sulfate reduction, this was later considered not to be the primary cause of gleying (Bloomfield, 1950). Waterlogged soil has been observed to bleach before sulfate reduction is detectable (Takai and Kamura, 1966). Bloomfield (1951) suggested that gleying was due at least in part to the action of products of plant decomposition, although he had earlier demonstrated gleying under artificial conditions in a sugar-containing medium that was allowed to ferment. Presently, microbial reduction of iron in which ferric iron is reduced by anaerobic bacterial respiration is favored as an explanation of gleying (Ottow, 1969a, 1970b, 1971). The reaction that iron undergoes in this microbial reduction has been summarized as follows (Ottow, 1971):

$$\text{Energy source} \xrightarrow[\text{dehydrogenase}]{\text{substrate}} e + H^+ + \text{ATP} + \text{end products} \tag{15.37}$$

$$Fe(OH)_3 + 3H^+ + e \rightarrow Fe^{2+} + 3H_2O \tag{15.38}$$

$$2Fe(OH)_3 + Fe^{2+} + 2OH^- \rightarrow \underset{\substack{\text{gray-green mixed}\\ \text{oxide of gley}}}{Fe_3(OH)_8} \tag{15.39}$$

15.12 MICROBES AND THE IRON CYCLE

Microbial transformations of iron play an important role in the cycling of iron in nature (Fig. 15.15). Weathering of iron-containing minerals in rocks, soils, and

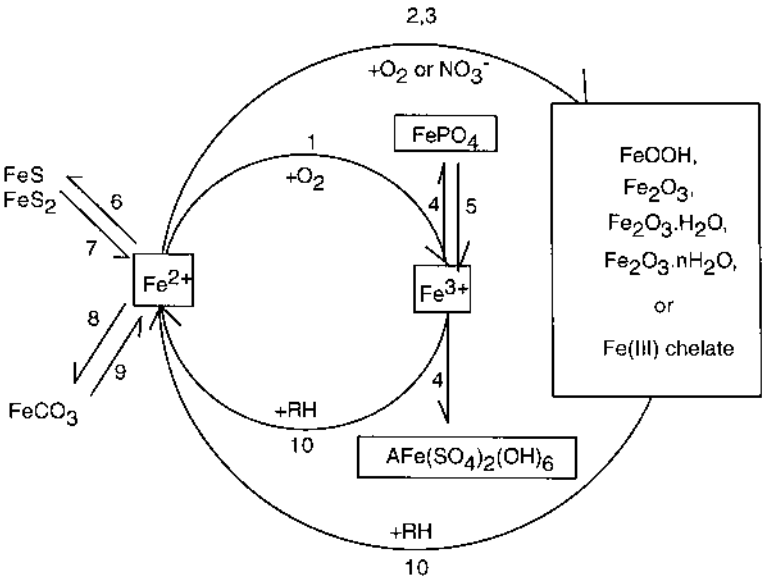


FIG. 15.15 The iron cycle. Numerical code: *1*, Microbially at acid pH; *2*, microbially at neutral pH in the dark with O_2 when O_2 tension is low, or anerobically in the dark with NO_3^-, or anaerobically in light (anoxygenic photosynthesis); *3*, chemically at neutral pH when O_2 tension is high; *4*, chemically; *5*, H^+, microbially; *6*, H_2S, often of microbial origin; *7*, $+O_2$, microbially or chemically; *8*, $+CO_3^{2-}$; *9*, H^+, microbially or chemically; *10*, microbially or chemically.

sediments introduces iron into the cycle. This weathering action is promoted partly by bacterial and fungal action and partly by chemical activity (e.g., Bloomfield, 1953a, 1953b; Lovley, 2000). The microbial action involves in many cases the interaction of the minerals with metabolic end products (Berthelin and Kogblevi, 1972, 1974; Berthelin and Dommergues, 1972; Berthelin et al., 1974). The mobilized iron, if ferrous, may be biologically or nonbiologically oxidized to ferric iron at a pH above 5 under partially or fully aerobic conditions independent of light. It can also be oxidized biologically under anaerobic conditions in light, i.e., photosynthetically, or in the presence of nitrate independent of light (see Sec. 15.3). At a pH below 4, ferrous iron is oxidized mainly biologically. The oxidation of ferrous to ferric iron may be followed by immediate precipitation of the ferric iron as a hydroxide, oxide, phosphate, or sulfate. If natural, soluble organic ligands abound, the ferric iron may be converted to soluble complexes and be dispersed from its site of formation. It may also be complexed by humic substances. In podzolic soils (spodosols) in temperate climates, complexed iron may be transported by groundwater from the upper A

horizon to the B horizon. In hot, humid climates, ferric iron is more likely to precipitate at the site of its formation after release from iron-containing soil minerals, owing to intense microbial activity that rapidly and fairly completely mineralizes available organic matter including ligands, which prevents extensive formation of soluble ferric iron complexes. The iron precipitates tend to cement soil particles together in a process known as **laterization**. Aluminum hydroxide liberated in the weathering process may also be precipitated and contribute to laterization (Brooks and Kaplan, 1972, p. 75; Merkle, 1955, p. 204; see also Chap. 10).

In freshwater environments, mobilization of iron may be prominent in sediments when the hypolimnion is oxygen-depleted. In sediment from Blelham Tarn in the English Lake District, bacteria occur that mineralize organic matter by iron respiration (Jones et al., 1983, 1984). Formation of Fe(II) by ferric iron reduction by a *Vibrio* of the bacterial flora in the sediment was stimulated by the addition of malate and inhibited by nitrate, MnO_2, and Mn_2O_3. H_2 was able to serve as a reductant (energy source) for the organism. Mineralization of the organic matter by iron respiration can be very important for the carbon cycle in some aquatic reducing environments (Lovley and Phillips, 1986a, 1987, 1988a). Reoxidation of mobilized ferrous iron by *Gallionella* has been reported to occur in the metalimnion of Hortlandsstemma, a small eutrophic lake in Norway where the dissolved oxygen concentration was only $0.005\,\text{mmol}\,L^{-1}$ at a pH of 6.5 (Heldal and Tumyr, 1983). At higher oxygen concentrations, the iron would have been autoxidized.

The microbial genesis of iron sulfides, including pyrite, at near neutral pH and the biological oxidation of iron sulfides at acid pH make very important contributions to the cycling of iron in environments such as salt marshes and sulfide ore deposits, respectively (see Chap. 19).

15.13 SUMMARY

Some bacteria can oxidize ferrous iron enzymatically with the generation of useful energy. Much of the evidence for this comes from the study of acidophilic iron oxidizers such as *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Sulfolobus* spp., *Acidianus brierleyi*, and *Sulfobacillus thermosulfidooxidans*. The first of these has been most extensively studied. The reason the most convincing evidence of microbial iron oxidation has come from the study of acidophiles is that ferrous iron is least susceptible to autoxidation below pH 5. Some bacteria growing at near-neutral pH can also oxidize ferrous iron enzymatically, but only under partially reduced conditions. The stalked bacterium *Gallionella ferruginea* uses the energy gained from this oxidation chemolithotrophically, whereas the sheathed bacteria *Leptothrix* spp. may use it mixotropically. *Leptothrix* may, in

addition, precipitate pre-existent ferric iron in the environment passively on their sheath.

Iron can also be oxidized nonenzymatically by microorganisms when they raise the redox potential and/or pH of their environment to levels that favor autoxidation. The rise in redox potential results from the buildup in the bulk phase of oxidants from their metabolism. The rise in pH is the consequence of photosynthesis, ammonia production, or the consumption of organic acids and/or their salts, some of which may chelate ferrous iron.

Ferric iron precipitation need not involve iron oxidation but rather the destruction of ferric iron chelates. Naturally occurring chelators that may solubilize extensive amounts of ferric iron include microbially produced oxalate, citrate, humic acids, and tannins. The resultant ferric chelates are relatively stable in solution unless the chelators are degraded (mineralized) by microbes. When such degradation occurs, ferric iron precipitation will result if the prevailing pH is circumneutral. A number of bacteria have this degradative capacity. Ferric iron may be locally concentrated by adsorption to cell surfaces. A variety of microorganisms, including bacteria and protozoa, have been found to have this capacity.

Microbial formation and accumulation of iron in aqueous environments may cause concurrent accumulation of other heavy metal ions by coprecipitation and adsorption to hydrous iron oxides (e.g., Gunkel, 1986). The adsorbed metals may be remobilized by reduction of the iron oxide or by acidification without reduction of the ferric adsorbent (Tipping et al., 1986).

Ferric iron can be enzymatically reduced to ferrous iron with a suitable electron donor. Nitrate reductase A is one enzyme that was implicated in early studies. However, at least one other enzyme appeared to be independently active in some nitrate reducers. Both bacteria and fungi have been implicated in ferric iron reduction by nitrate reductase. Currently, a variety of facultative and strictly anaerobic bacteria have been found that can use ferric iron as terminal electron acceptor under anaerobic conditions. In at least one of the gram-negative bacteria involved, the ferric iron reductase appears to be c-type cytochrome or a protein associated with it in the outer membrane. Many of the ferric iron reducers are heterotrophs that mineralize the organic carbon that they use as electron donor, whereas others degrade this carbon only incompletely. Some can use H_2 as electron donor. A few chemoautotrophs have been found to be able to use ferric iron in place of oxygen as terminal electron donor. The ferric iron–respiring bacteria can play an important role in promoting the carbon cycle anaerobically.

Not all microbial ferric iron reduction is enzymatic. Some ferric iron reduction can be the result of reduction by products of microbial metabolism such as H_2S or formate.

Both oxidative and reductive reactions involving iron that are brought about by microbes play important roles in the iron cycle in nature. They affect the

mobility of iron as well as its local accumulation. The formation of some sedimentary iron deposits has been attributed directly to microbial activity. The formation of ochre and bog and lake iron has been associated with bacterial iron oxidation. Gleying has been associated with bacterial iron reduction, as has the formation of magnetite and siderite. Ferric iron respiration can be a more important mechanism of carbon mineralization in some anaerobic environments than sulfate respiration.

REFERENCES

Adams LF, Ghiorse WC. 1987. Characterization of extracellular Mn^{2+}-oxidizing activity and isolation of Mn^{2+}-oxidizing protein from *Leptothrix discophora* SS-1. J Bacteriol 169:1279–1285.

Agate AD, Vishniac W. 1970. Transport characteristics of phospholipids from thiobacilli. Bacteriol Proc GP14:50.

Ahonen L, Tuovinen OH. 1989. Microbial oxidation of ferrous iron at low temperature. Appl Environ Microbiol 55:312–316.

Aleem MIH, Lees H, Nicholas DJD. 1963. Adenosine triphosphate-dependent reduction of nicotinamide adenine dinucleotide by ferro-cytochrome c in chemoautotrophic bacteria. Nature (Lond) 200:759–761.

Alexander M. 1977. Introduction to Soil Microbiology. 2nd ed. New York: Wiley.

Anbar AD, Holland HD. 1992. The photochemistry of manganese and the origin of banded iron formations. Geochim Cosmochim Acta 56:2595–2603.

Anderson KJ, Lundgren DG. 1969. Enzymatic studies of the iron-oxidizing bacterium, *Ferrobacillus ferrooxidans*: Evidence for a glycolytic pathway and Krebs cycle. Can J Microbiol 15:73–79.

Aristovskaya TV, Zavarzin GA. 1971. Biochemistry of iron in soil. In: McLaren AD, Skujins J, eds. Soil Biochemistry, Vol 2. New York: Marcel Dekker, pp 385–408.

Arkesteyn GJMW. 1979. Pyrite oxidation of *Thiobacillus ferrooxidans* with special reference to the sulfur moiety of the mineral. Antonie v Leeuwenhoek 45:423–435.

Arkesteyn GJMW, DeBont JAM. 1980. *Thiobacillus acidophilus*: A study of its presence in *Thiobacillus ferrooxidans* cultures. Can J Microbiol 26:1057–1065.

Arnold RG, DiChristina TJ, Hoffmann MR. 1986a. Inhibitor studies of dissimilative Fe(III) reduction by *Pseudomonas* sp. strain 200 ("*Pseudomonas ferrireductans*"). Appl Environ Microbiol 52:281–289.

Arnold RG, Olson TM, Hoffmann MR. 1986b. Kinetics and mechanism of dissimilative Fe(III) reduction by *Pseudomonas* sp. 200. Biotech Bioeng 28:1657–1671.

Arnold RG, Hoffmann MR, DiChristina TJ, Picardal FW. 1990. Regulation of dissimilatory Fe(III) reduction activity in *Shewanella putrefaciens*. Appl Environ Microbiol 56:2811–2817.

Atlas RM. 1997. Principles of Microbiology. 2nd ed. Boston, MA: WCB McGraw-Hill.

Avakyan AA, Karavaiko GI. 1970. Submicroscopic organization of *Thiobacillus ferrooxidans*. Mikrobiologiya 39:855–861 (Engl transl, pp 744–751).

Baas Becking LGM, Parks GS. 1927. Energy relations in the metabolism of autotrophic bacteria. Physiol Rev 7:85–106.

Balashova VV, Zavarzin GA. 1972. Iron oxidation by *Mycoplasma laidlawii*. Mikrobiologiya 41:909–911 (Engl transl, pp 808–810).

Balashova VV, Zavarzin GA. 1979. Anaerobic reduction of ferric iron by hydrogen bacteria. Mikrobiologiya 48:773–778 (Engl transl, pp 635–639).

Balashova VV, Vedina IYa, Markosyan GE, Zavarzin GA. 1974. *Leptospirillum ferrooxidans* and aspects of its autotrophic growth. Mikrobiologiya 43:581–585 (Engl transl, pp 491–494).

Balkwill DL, Maratea D, Blakemore RP. 1980. Ultrastructure of a magnetotactic bacterium. J Bacteriol 141:1399–1408

Barker WW, Welch SA, Chu S, Banfield JF. 1998. Experimental observations of the affects of bacteria on aluminosilicate weathering. Am Mineral 83:1551–1563.

Barros MEC, Rawlings DE, Woods DR. 1984. Mixotrophic growth of the *Thiobacillus ferrooxidans* strain. Appl Environ Microbiol 47:593–595.

Bazylinski DA, Frankel RB. 2000. Biologically controlled mineralization of magnetic iron minerals by magnetotactic bacteria. In: Lovley DR, ed. Environmental Microbe-Metal Interactions. Washington, DC: ASM Press, pp 109–144.

Beck JV. 1960. A ferrous-ion oxidizing bacterium. I. Isolation and some general physiological characteristics. J Bacteriol 79:502–509.

Beck JV, Elsden SR. 1958. Isolation and some characteristics of an iron-oxidixing bacterium. J Gen Microbiol 19, i.

Bell PE, Mills AL, Herman JS. 1987. Biogeochemical conditions favoring magnetite formation during anaerobic iron reduction. Appl Environ Microbiol 53:2610–2616.

Benz M, Brune A, Schink B. 1998. Anaerobic and aerobic oxidation of ferrous iron at neutral pH by chemoheterotrophic nitrate-reducing bacteria. Arch Microbiol 169:159–165.

Berner RA. 1962. Experimental studies of the formation of sedimentary iron sulfides. In: Jensen ML, ed. Biogeochemistry of Sulfur Isotopes. New Haven, CT: Yale Univ Press, pp 107–120.

Berry VK, Murr LE. 1980. Morphological and ultrastructural study of the cell envelope of thermophilic and acidophilic microorganisms as compared to *Thiobacillus ferrooxidans*. Biotech Bioeng 22:2543–2555.

Berthelin J, Dommergues Y. 1972. Rôle de produits du métabolisme microbien dans la solubilization des minéraux d'une arène granitique. Rev Ecol Biol Sol 9:397–406.

Berthelin J, Kogblevi A. 1972. Influence de la stérilization partielle sur la solubilization microbienne des minéraux dans les sols. Rev Ecol Biol 9:407–419.

Berthelin J, Kogblevi A. 1974. Influence de l'engorgement sur l'altération microbienne des minéraux dans les sols. Rev Ecol Biol 11:499–509.

Berthelin J, Kogblevi A, Dommergues Y. 1974. Microbial weathering of a brown forest soil: Influence of partial sterilization. Soil Biol Biochem 6:393–399.

Blake R II, Waskovsky J, Harrison AP Jr. 1992. Respiratory components in acidophilic bacteria that respire on iron. Geomicrobiol J 10:173–192.

Blake RC, Shute EA. 1987. Respiratory enzymes of *Thiobacillus ferrooxidans*. J Biol Chem 262:14983–14989.

Blake RC, Shute EA, Greenwood MM, Spencer GH, Ingledew WJ. 1993. Enzymes of aerobic respiration on iron. FEMS Microbiol Rev 11:9–18.

Blakemore RP. 1982. Magnetotactic bacteria. Annu Rev Microbiol 36:217–238.

Blakeney MD, Moulaei T, DiChristina TJ. 2000. Fe(III) reduction activity and cytochrome content of *Shewanella putrefaciens* grown on ten compounds as sole terminal electron acceptor. Microbiol Res 155:87–94.

Blaylock BA, Nason A. 1963. Electron transport systems of the chemoautotroph *Ferrobacillus ferrooxidans*. 1. Cytochrome c-containing iron oxidase. J Biol Chem 238:3453–3462.

Bloomfield C. 1950. Some observations on gleying. J Soil Sci 1:205–211.

Bloomfield C. 1951. Experiments on the mechanism of gley formation. J Soil Sci 2:196–211.

Bloomfield C. 1953a. A study of podzolization. Part I. The mobilization or iron and aluminum by Scots pine needles. J Soil Sci 4:5–16.

Bloomfield C. 1953b. A study of podzolization. Part II. The mobilization of iron and aluminum by the leaves and bark of Agathis australis (Kauri). J Soil Sci 4:17–23.

Boone DR, Liu Y, Zhao Z-J, Balkwill DL, Drake GR, Stevens TO, Aldrich HC. 1995. *Bacillus infernus* sp. nov., an Fe(III)- and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. Int J Syst Bacteriol 45:441–448.

Bridge TAM, Johnson DB. 1998. Reduction of soluble iron and reductive dissolution of ferric iron containing minerals by moderately thermophilic iron-oxidizing bacteria. Appl Environ Microbiol 64:2181–2186.

Bridge TAM, Johnson DB. 2000. Reductive dissolution of ferric iron minerals by Acidiphilium SJH. Geomicrobiol J 17:193–206.

Brierley CL, Brierley JA. 1973. A chemoautotrophic microorganism isolated from an acid hot spring. Can J Microbiol 19:183–188.

Brierley CL, Murr LE. 1973. Leaching: Use of a thermophilic and chemoautotrophic microbe. Science 179:488–499.

Brock TD, Gustafson J. 1976. Ferric iron reduction by sulfur- and iron-oxidizing bacteria. Appl Environ Microbiol 32:567–571.

Brock TD, Brock KM, Belly RT, Weiss RL. 1972. *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch Microbiol 84:54–68.

Brock TD, Cook S, Peterson S, Mosser JL. 1976. Biogeochemistry and bacteriology of ferrous iron oxidation in geothermal habitats. Geochim Cosmochim Acta 40:493–500.

Bromfield SM. 1954a. The reduction of iron oxide by bacteria. J Soil Sci 5:129–139.

Bromfield SM. 1954b. Reduction of ferric compounds by soil bacteria. J Gen Microbiol 11:1–6.

Brooks RR, Kaplan IR. 1972. Biogeochemistry. In: The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 74–82.

Brown KA, Ratledge C. 1975. Iron transport in *Mycobacterium smegamtis*: Ferrimycobactin reductase (NAD(P)H : ferrimycobactin oxidoreductase), the enzyme releasing iron from its carrier. FEBS Lett 53:262–266.

Buchanan RE, Gibbons NE, eds. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore, MD: Williams & Wilkins.

Burdige DJ. 1993. The biogeochemistry of manganese and iron reduction in marine sediments. Earth-Sci Rev 35:249–284.

Caccavo F Jr, Blakemore RP, Lovley DR. 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. Appl Environ Microbiol 58:3211–3216.

Caccavo F Jr, Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ. 1994. *Geobacter sulfurreducens* sp. nov., hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol 60:3752–3759.

Caccavo F Jr, Coates JD, Rossello-Mara RA, Ludwig W, Schleifer KH, Lovley DR, McInerney MJ. 1996. *Geovibrio ferrireducens*, a phylogenetically distinct dissimilatory Fe(III)-reducing bacterium. Arch Microbiol 165:370–376.

Champine JE, Goodwin S. 1991. Acetate catabolism in the dissimilatory iron-reducing isolate GS-15. J Bacteriol 173:2704–2706.

Champine JE, Underhill B, Johnston JM, Lilly WW, Goodwin S. 2000. Electron transfer in the dissimilatory iron-reducing bacterium *Geobacter metallireducens*. Anaerobe 6:187–196.

Cholodny N. 1924. Zur Morphologie der Eisenbakterien *Gallionella* and *Spirophyllum*. Ber deutsch. Bot Ges 42:35–44.

Cholodny N. 1926. Die Eisenbakterien. Beiträge zu einer Monographie. Jena, Germany: Verlag von Gustav Fischer.

Clark DA, Norris PR. 1996. *Acidimicrobium ferrooxidans* gen nov., sp. nov.: Mixed culture ferrous iron oxidation with *Sulfobacillus* species. Microbiology (Reading) 142:785–790.

Cloud PE Jr. 1973. Paleoecological significance of the banded-iron formations. Econ Geol 68:1135–1143.

Cloud PE Jr, Licari GR. 1968. Microbiotas of the banded iron formations. Proc Natl Acad Sci, USA 61:779–786.

Coates JD, Ellis DJ, Gaw CV, Lovley DR. 1999. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon contaminated aquifer. Int J Syst Bacteriol 49:1615–1622.

Cobley JG, Haddock BA. 1975. The respiratory chain of *Thiobacillus ferrooxidans*: The reduction of cytochromes by Fe^{2+} and the preliminary characterization of rusticyanin, a novel "blue" copper protein. FEBS Lett 60:33.

Coleman ML, Hedrick DB, Lovley DR, White DC, Pye K. 1993. Reduction of Fe(III) in sediments by sulfate-reducing bacteria. Nature (Lond) 361:436–438.

Colmer AR, Temple KL, Hinkle HE. 1950. An iron-oxidizing bacterium from the acid drainage of some bituminous coal mines. J Bacteriol 59:317–328.

Corbett CM, Ingledew WJ. 1987. Is Fe^{3+}/Fe^{2+} cycling an intermediate in sulfur oxidation by Fe^{2+}-grown *Thiobacillus ferrooxidans*? FEMS Microbiol Lett 41:1–6.

Corstjens PLAM, de Vrind JPM, Westbroek P, de Vrind-de Jong EW. 1992. Enzymatic iron oxidation by *Leptothrix discophora*: Identification of an iron-oxidizing protein. Appl Environ Microbiol 58:450–454.

Cox CD. 1980. Iron reductases from *Pseudomonas aeruginosa*. J Bacteriol 141:199–204.

Cox JC, Boxer DH. 1978. The purification and some properties of rusticyanin, a blue copper protein involved in iron(II) oxidation from *Thiobacillus ferrooxidans*. Biochem J 174:497–502.

Cox JC, Boxer DH. 1986. The role of rusticyanin, a blue copper protein, in the electron transport chain of *Thiobacillus ferrooxidans* grown on iron or thiosulfate. Biotech Appl Biochem 8:269–275.

Crerar DA, Knox GW, Means JL. 1979. Biogeochemistry of bog iron in the New Jersey Pine Barrens. Chem Geol 24:111–135.

Cummings DE, Caccavo F Jr, Spring S, Rosenzweig RF. 1999. *Ferribacterium limneticum*, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining impacted freshwater lake sediments. Arch Microbiol 171:183–188.

De Castro AF, Ehrlich HL. 1970. Reduction of iron oxide minerals by a marine *Bacillus*. Antonie v Leeuwenhoek 36:317–327.

de Vrind-de Jong, EW, Corstjens PLAM, Kempers ES, Westbroek P, de Vrind JPM. 1990. Oxidation of manganese and iron by *Leptothrix discophora*: Use of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine as an indicator of metal oxidation. Appl Environ Microbiol 56:3458–3462.

Din GA, Suzuki I, Lees H. 1967a. Ferrous iron oxidation by *Ferrobacillus ferrooxidans*. Purification and properties of Fe^{2+}-cytochrome c reductase. Can J Biochem 45:1523–1546.

Din GA, Suzuki I, Lees H. 1967b. Carbon dioxide and phosphoenolpyruvate carboxylase in *Ferrobacillus ferrooxidans*. Can J Microbiol 13:1413–1419.

Dobbin PS, Carter JP, San Juan G-SC, von Hobe M, Powell AK, Richardson DJ. 1999. Dissimilatory Fe(III) reduction by *Clostridium beijerinckii* isolated from freshwater sediment using Fe(III) maltol enrichment. FEMS Microbiol Lett 176:131–138.

Drobner E, Huber H, Stetter KO. 1990. *Thiobacillus ferrooxidans*, a facultative hydrogen oxidizer. Appl Environ Microbiol 56:2922–2923.

Dubinina GA. 1978a. Mechanism of the oxidation of divalent iron and manganese by iron bacteria growing at neutral pH of the medium. Mikrobiologiya 47:591–599 (Engl transl, pp 471–478).

Dubinina GA. 1978b. Functional role of bivalent iron and manganese oxidation in *Leptothrix pseudoochracea*. Mikrobiologiya 47:783–789 (Engl transl, pp 631–639).

Dugan PR, Lundgren DG. 1965. Energy supply for the chemoautotroph *Ferrobacillus ferrooxidans*. J Bacteriol 89:825–834.

Edwards, KJ, Bond PL, Gihrig TM, Bandield JF. 2000. An Archaeal iron- oxidizing extreme acidophile important in acid mine drainage. Science 287:1796–1799.

Ehrenberg CG. 1836. Vorläufige Mitteilungen über das wirkliche Vorkommen fossiler Infusorien und ihre gross Verbreitung. Poggendorf's Ann Phys Chem 38:213–227.

Ehrenreich A, Widdel F. 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. Appl Environ Microbiol 60:4517–4526.

Ehrlich HL. 1993. Bacterial mineralization of organic carbon under anaerobic conditions. In: Bollag J-M, Stotzky G, eds. Soil Biochemistry, Vol. 8. New York: Marcel Dekker, pp 219–247.

Ehrlich HL, Yang SH, Mainwaring JD. 1973. Bacteriology of manganese nodules. VI. Fate of copper, nickel, cobalt, and iron during bacterial and chemical reduction of the manganese (IV). Z Allg Mikrobiol 13:39–48.

Elbehti A, Brasseur G, Lemesle-Meunier D. 2000. First evidence for existence of an uphill electron transfer through the bc_1 and NADH-Q oxidoreductase complexes of the

acidophilic obligate chemolithotrophic ferrous ion-oxidizing bacterium *Thiobacillus ferrooxidans*. J Bacteriol 182:3602–3606.

Emerson D. 2000. Microbial oxidation of Fe(II) and Mn(II) at circumneutral pH. In: Lovley DR, ed. Environmental Microbe-Metal Interactions. Washington, DC: ASM Press, pp 31–52.

Emerson D, Ghiorse WC. 1993a. Ultrastructure and chemical composition of the sheath of *Leptothrix discophora* SP-6. J Bacteriol 175:7808–7818.

Emerson D, Ghiorse WC. 1993b. Role of disulfide bonds in maintaining the structural integrity of the sheath of *Leptothrix discophora* SP-6. J Bacteriol 175:7819–7827.

Ernst JG, Winkelmann G. 1977. Enzymatic release of iron from sideramines in fungi. NADH : sideramine oxidoreductase in *Neurospora crassa*. Biochim Biophys Acta 500:27–41.

Ernstsen V, Gates WP, Stucki JW. 1998. Microbial reduction of structural iron in clays: A renewable resource of reduction capacity. J Environ Qual 27:761–766.

Fischer J, Quentmeister A, Kostka S, Kraft R, Friedrich CG. 1996. Purification and characterization of the hydrogenase from *Thiobacillus ferrooxidans*. Arch Microbiol 165:289–296.

Frankel RB, Papaefthymiou GC, Blakemore RP, O'Brien W. 1983. Fe_3O_4 precipitation in magnetotactic bacteria. Biochim Biophys Acta 763:147–159.

Fry IV, Lazaroff N, Packer L. 1986. Sulfate-dependent iron oxidation by *Thiobacillus ferrooxidans*: Characterization of a new EPR detectable electron transport component on the reducing side of rusticyanin. Arch Biochem Biophys 246:650–654.

Fukumori Y, Yano T, Sato A, Yamanaka T. 1988. Fe(II)-oxidizing enzyme purified from *Thiobacillus ferrooxidans*. FEMS Microbiol Lett 50:169–172.

Gale NL, Beck JV. 1967. Evidence for the Calvin cycle and hexose monophosphate pathway in *Thiobacillus ferrooxidans*. J Bacteriol 94:1052–1059.

Gelder RJ. 1999. Complex lessons of iron uptake. Nature (Lond) 400:815–816.

Ghiorse WC. 1984. Biology of iron- and manganese-depositing bacteria. Annu Rev Microbiol 38:515–550.

Ghiorse WC. 1988. Microbial reduction of manganese and iron. In: Zehnder AJB, ed. Biology of Anaerobic Microorganisms. New York: Wiley, pp 305–331.

Ghiorse WC, Ehrlich HL. 1992. Microbial biomineralization of iron and manganese. In: Skinner HCW, Fitzpatrick RW, eds. Biomineralization. Process of Iron and Manganese. Modern and Ancient Environments. Catena Suppl 21. Cremlingen, Germany: Catena Verlag, pp 75–99.

Gibson F, Magrath DI. 1969. The isolation and characterization of a hydroxamic acid (aerobactin) formed by *Aerobacter aerogenes* 62-1. Biochim Biophys Acta 192:175–184.

Golovacheva RS, Karavaiko GI. 1978. A new genus of thermophilic spore-forming bacteria, *Sulfobacillus*. Mikrobiologiya 47:815–822 (Engl transl, pp 658–665).

Golyshina OV, Pivovarova TA, Karavaiko GI, Kondrat'eva TF, Moore ERB, Abraham WR, Lunsdorf H, Timmis KN, Yakimov MM, Golyshin PM. 2000. *Ferroplasma acidiphilium* gen. nov., spec. nov., an acidophilic, autotrophic, ferrous-iron oxidizing, cell-wall lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the Archaea. Int J Syst Evol Microbiol 50:997–1006.

Gómez JM, Cantero D, Johnson DB. 1999. Comparison of the effects of temperature and pH on iron oxidation and survival by *Thiobacillus ferrooxidans* (type strain) and a "*Leptospirillum ferrooxidans*"-like isolate. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century. Part A. Amsterdam: Elsevier, pp 689–696.

González-Toril E, Gómez N, Izabal R, Amils R, Marín I. 1999. Comparative genomic characterization of iron oxidizing bacteria isolated from the Tinto River. In: Amils R, Ballester A, eds. *Biohydrometallurgy and the Environment Toward the Mining of the 21st Century.* Part B. Amsterdam: Elsevier, pp 149–157.

Gorby YA, Lovley DR. 1991. Electron transport in the dissimilatory iron reducer, GS-15. Appl Environ Microbiol 57:867–870.

Gorby YA, Beveridge TJ, Blakemore RP. 1988. Characterization of the bacterial magnetosome membrane. J Bacteriol 170:834–841.

Gruner JW. 1923. Algae, believed to be Archean. J Geol 31:146–148.

Guay R, Silver M. 1975. *Thiobacillus acidophilus* sp. nov. Isolation and some physical characteristics. Can J Microbiol 21:281–288.

Gunkel G. 1986. Studies of the fate of heavy metals in lakes. I. The role of ferrous oxidizing bacteria in coprecipitation of heavy metals. Arch Hydrobiol 105:489–515.

Hafenbrandl D, Keller M, Dirmeier R, Rachel R, Rossnagel P, Burggraf S, Huber H, Stetter KO. 1996. *Ferroglobus placidus* gen nov., spec. nov., a novel hyperthermophilic archeum that oxidizes Fe^{2+} at neutral pH under anoxic conditions. Arch Microbiol 166:308–314.

Hallbeck L. 1993. On the biology of the iron-oxidizing and stalk-forming bacterium *Gallionella ferruginea*. PhD Thesis. University of Göteborg, Sweden.

Hallbeck L., Pedersen K. 1990. Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. J Gen Microbiol 136:1675–1680.

Hallbeck L., Pedersen K. 1991. Autotrophic and mixotrophic growth of *Gallionella ferruginea*. J Gen Microbiol 137:2657–2661.

Hammann R, Ottow JCG. 1974. Reductive dissolution of Fe_2O_3 by saccharolytic Clostridia and *Bacillus polymyxa* under anaerobic conditions. Z Pflanzenernähr Bodenk 137:108–115.

Hanert H. 1968. Untersuchungen zur Isolierung, Stoffwechselphysiologie und Morphologie von *Gallionella ferruginea* Ehrenberg Arch Mikrobiol 60:348–376.

Hanert H. 1973. Quantifizierung der Massenentwicklung des Eisenbakteriums *Gallionella ferruginea* unter natürlichen Bedingungen. Arch Mikrobiol 88:225–243.

Hanert H. 1974a. Untersuchungen zur individuellen Entwicklungskinetik von *Gallionella ferruginea* in statischer Mikrokultur. Arch Mikrobiol 96:59–74.

Hanert H. 1974b. In situ Untersuchungen zur Analyse und Intensität der Eisen(III)-Fällung in Dränungen. Z Kulturtech Flurberein 15:80–90.

Hanert HH. 1981. The genus *Gallionella*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, eds. The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria. Berlin: Springer-Verlag, pp 509–515.

Harder EC. 1919. Iron depositing bacteria and their geologic relations. US Geol Surv Prof Pap 113.

Harrison AP Jr. 1981. *Acidiphilium cryptum* gen. nov., spec. nov., heterotrophic bacterium from acidic mineral environments. Int J Syst Bacteriol 31:327–332.

Harrison AP Jr. 1982. Genomic and physiological diversity amongst strains of *Thiobacillus ferrooxidans* and genomic comparison with *Thiobacillus thiooxidans*. Arch Microbiol 131:68–76.

Harrison AP Jr. 1984. The acidophilic thiobacilli and other acidophilic bacteria that share their habitat. Annu Rev Microbiol 38:265–292.

Harrison AP Jr. 1986. The phylogeny of iron-oxidizing bacteria. In: Ehrlich HL, Holmes DS, eds. *Workshop on Biotechnology for the Mining, Metal-Refining and Fossil Fuel Processing Industries*. Biotech Bioeng Symp 16. New York: Wiley, pp 311–317.

Harrison AP Jr., Norris PR. 1985. *Leptospirillum ferrooxidans* and similar bacteria: Some characteristics and genomic diversity. FEMS Microbiol Lett 30:99–102.

Hart A, Murrell JC, Poole RK, Norris PR. 1991. An acid-stable cytochrome in iron-oxidizing *Leptospirillum ferrooxidans*. FEMS Microbiol Lett 81:89–94.

Heldal M, Tumyr O. 1983. *Gallionella* from metalimnion in a eutrophic lake: Morphology, X-ray energy-dispersive microanalysis of apical cells and stalks. Can J Microbiol 29:303–308.

Hersman LE, Huang A, Maurice PA, Forsythe JH. 2000. Siderophore production and iron reduction by *Pseudomonas mendocina* in response to iron deprivation. Geomicrobiol J 17:261–273.

Hilgenfeldt K. 2000. Diagenetic dissolution of biogenic magnetite in surface sediments of the Benguela upwelling system. Int J Earth Sci 88:630–640.

Hoehnl G. 1955. Ein Beitrag zur Physiologie der Eisenbakterien. Vom Wasser 22:176–193.

Holm NG. 1987. Biogenic influence on the geochemistry of certain ferruginous sediments of hydrothermal origin. Chem Geol 63:45–57.

Holmes DS, Lobos JH, Bopp LH, Welch GC. 1983. Setting up a genetic system de novo for studying the acidophilic thiobacillus *T. ferrooxidans*. In: Rossi G, Torma AE, eds. Recent Progress in Biohydrometallurgy. Iglesias, Italy: Associazione Mineraria Sarda, pp 541–554.

Holmes DS, Yates JR, Schrader J. 1989. Mobile repeated DNA sequences in *Thiobacillus ferrooxidans* and their significance for biomining. In: Norris PR, Kelly DP, eds. Biohydrometallurgy. Kew, Surrey, UK: Sci Technol Lett, pp 153–160.

Holmes DS, Zhao H-L, Levican G, Ratouchniak J, Bonnefoy V, Varela P, Jedlicki E. 2001. ISAfe 1, an ISL3 family insertion sequence from *Acidithiobacillus ferrooxidans* ATCC 19859. J Bacteriol 183:4323–4329.

Huber H, Stetter KO. 1989. *Thiobacillus prosperus* sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. Arch Microbiol 151:479–485.

Huber R, Kristjansson JK, Stetter KO. 1987. *Pyrobaculum* gen nov., a new genus of neutrophilic, rod-shaped archaebacteria from continental solfataras growing optimally at 100°C. Arch Microbiol 149:95–101.

Hutchinson M, Johnstone KI, White D. 1966. Taxonomy of the acidophilic Thiobacilli. J Gen Microbiol 44:373–381.

Ingledew WJ. 1982. *Thiobacillus ferrooxidans*. The bioenergetics of an acidophilic chemolithotroph. Biochem Biophys Acta 683:89–117.

Ingledew WJ. 1986. Ferrous iron oxidation by *Thiobacillus ferrooxidans*. In: Ehrlich HL, Holmes DS, eds. Workshop on Biotechnology for the Mining, Metal-Refining and Fossil Fuel Processing Industries. Biotech Bioeng Symp 16. New York: Wiley, pp 23–33.

Ingledew WJ, Houston A. 1986. The organization of the respiratory chain of *Thiobacillus ferrooxidans*. Biotech Appl Biochem 8:242–248.

Ingledew WJ, Cox JC, Halling PJ. 1977. A proposed mechanism for energy conservation during Fe^{2+} oxidation by *Thiobacillus ferrooxidans*: Chemiosmotic coupling to net H^+ influx. FEMS Microbiol Lett 2:193–197.

Ivanov MV, Lyalikova NN. 1962. Taxonomy of iron-oxidizing thiobacilli. Mikrobiologiya 31:468–469 (Engl transl, pp 382–383).

Ivarson KC, Sojak M. 1978. Microorganisms and ochre deposition in field drains of Ontario. Can J Soil Sci 58:1–17.

Johnson DB, Kelso WI. 1983. Detection of heterotrophic contaminants in cultures of *Thiobacillus ferrooxidans* and their elimination by subculturing in media containing copper sulfate. J Gen Microbiol 129:2969–2972.

Johnson DB, McGinness S. 1991. A highly efficient and universal solid medium for growing mesophilic and moderately thermophilic iron-oxidizing acidophilic bacteria. J Microbiol Methods 13:113–122.

Johnson DB, Roberto FF. 1997. Heterotrophic acidophiles and their roles in bioleaching of sulfide minerals. In: Rawlings DE, ed. Biomining: Theory, Microbes and Industrial Processes. Berlin: Springer-Verlag, pp 259–279.

Johnson DB, Macvicarl JHM, Rolfe S. 1987. A new solid medium for isolation and enumerations of *Thiobacillus ferrooxidans* and acidophilic heterotrophic bacteria. J Microbiol Methods 7:9–18.

Johnson DB, Said MF, Ghauri MA, McGinness S. 1989. Isolation of novel acidophiles and their potential use in bioleaching operations. In: Salley J, McCready RGL, Wichlacz PL, eds. Biohydrometallurgy. Proc Int Symp, Jackson Hole, WY, Aug 13–18, 1989. CANMET SP89-10, Ottawa, ON: Canada Centre for Mineral and Energy Technology, pp 403–414.

Johnson DB, Ghauri MA, Said MF. 1993. Isolation and characterization of an acidophilic, heterotrophic bacterium capable of oxidizing ferrous iron. Appl Environ Microbiol 58:1423–1428.

Jones CA, Kelly DP. 1983. Growth of *Thiobacillus ferrooxidans* on ferrous iron in chemostat culture: Influence of product and substrate inhibition. J Chem Tech Biotechnol 33B:241–261.

Jones JG, Gardener S, Simon BM. 1983. Bacterial reduction of ferric iron in a stratified eutrophic lake. J Gen Microbiol 129:131–139.

Jones JG, Gardener S, Simon BM. 1984. Reduction of ferric iron by heterotrophic bacteria in lake sediments. J Gen Microbiol 130:45–51.

Kamalov MR. 1967. Oxidation of divalent iron by *Thiobacillus ferrooxidans* culture in the presence of chloride and sulfate ions. Izv Akad Nauk Kaz SSR Ser Biol 5:47–50.

Kamalov MR. 1972. Adaptation of *Thiobacillus ferrooxidans* cultures to increased amounts of copper, zinc, and molybdenum in an acid medium. Izv Akad Nauk Kaz Ser Biol 10:39–44.

Karavaiko GI, Avakyan AA. 1970. Mechanisms of *Thiobacillus ferrooxidans* multiplication. Mikrobiologiya 39:950–952.

Karlin R, Lyle M, Heath GR. 1987. Authigenic magnetite formation in suboxic marine sediments. Nature (Lond) 326:490–493.

Kashefi K, Lovley DR. 2000. Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. Appl Environ Microbiol 66:1050–1056.

Kelly DP, Jones CA. 1978. Factors affecting metabolism and ferrous iron oxidation in suspensions and batch cultures of *Thiobacillus ferrooxidans*: Relevance to ferric iron leach solution generation. In: Murr LE, Torma AE, Brierley JA, eds. Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena. New York: Academic Press, pp 19–44.

Kelly DP, Tuovinen OH. 1972. Recommendation that the names *Ferrobacillus ferrooxidans* Leathen and Braley and *Ferrobacillus sulfooxidans* Kinsel be recognized as synonymous of *Thiobacillus ferrooxidans* Temple and Colmer. Int J Syst Bacteriol 22:170–172.

Kelly DP, Wood AP. 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov., and *Thermithiobacilllus* gen. nov. Int J Syst Evol Microbiol 50:511–516.

Kinsel N. 1960. New sulfur oxidizing iron bacterium; *Ferrobacillus sulfooxidans* sp. n. J Bacteriol 80:628–632.

Knight V, Blakemore R. 1998. Reduction of diverse electron acceptors by *Aeromonas hydrophila*. Arch Microbiol 169:239–248.

Konhauser KO. 1997. Bacterial iron biomineralization in nature. FEMS Microbiol Rev 20:315–326.

Kostka JE, Nealson KH. 1995. Dissolution and reduction of magnetite by bacteria. Environ Sci Technol 29:2535–2540.

Kostka JE, Stucki JW, Nealson KH, Wu J. 1996. Reduction of structural Fe(III) in smectite by a pure culture of *Shewanella putrefaciens* strain MR-1. Clays Clay Miner 44:522–529.

Kovalenko TV, Karavaiko GI, Piskunov VP. 1982. Effect of Fe^{3+} ions on the oxidation of ferrous iron by *Thiobacillus ferrooxidans* at various temperatures. Mikrobiologiya 51:156–160 (Engl transl, pp 142–146).

Krul IM, Hirsch P, Staley JT. 1970. *Toxothrix trichogenes* (Mol) Berger and Bingmann: The organism and its biology. Antonie v Leeuwenhoek 36:409–420.

Kucera S, Wolfe RS. 1957. A selective enrichment method for *Gallionella ferruginea*. J Bacteriol 74:344–349.

Kullman K-H, Schweisfurth R. 1978. Eisenoxydierende, stäbchenförmige Bakterien. II. Quantitative Untersuchungen zum Stoffwechsel und zur Eisoxydation mit Eisen(II)-oxalat. Z Allg Mikrobiol 18:321–327.

Kusano T, Sugawara K, Inoue C, Takeshima T, Numata M, Shiratori T. 1992. Electrotransformation of *Thiobacillus ferrooxidans* with plasmids containing a *mer* determinant as the selective marker by electroporation. J Bacteriol 174:6617–6623.

Kuznetsov SI, Romanenko VI. 1963. The Microbiological Study of Inland Waters. Moscow: Izvddatel'stvo Akad Nauk SSSR.

Landesman J, Duncan DW, Walden CC. 1966. Iron oxidation by washed cell suspensions of the chemoautotroph *Thiobacillus ferrooxidans*. Can J Microbiol 12:25–33.

Lascelle J, Burke KA. 1978. Reduction of ferric iron by L-lactate and DL-glycerol-3-phosphate in membrane preparations from *Staphylococcus aureus* and interactions with the nitrate reductase system. J Bacteriol 134:585–589.

Laverman AM, Switzer Blum J, Schaefer JK, Phillips EJP, Lovley DR, Oremland RS. 1995. Growth of strain SES-3 with arsenate and other diverse electron acceptors. Appl Environ Microbiol 61:3556–3561.

Lazaroff N. 1963. Sulfate requirement for iron oxidation by *Thiobacillus ferrooxidans*. J Bacteriol 85:78–83.

Lazaroff N. 1977. The specificity of the anionic requirement for iron oxidation by *Thiobacillus ferrooxidans*. J Gen Microbiol 101:85–91.

Lazaroff N. 1983. The exclusion of D_2O from the hydration sphere of $FeSO_4 \cdot 7H_2O$ oxidized by *Thiobacillus ferrooxidans*. Science 222:1331–1334.

Lazaroff N, Sigal W, Wasserman A. 1982. Iron oxidation and precipitation of ferric hydroxysulfates by resting *Thiobacillus ferrooxidans* cells. Appl Environ Microbiol 43:924–938.

Lazaroff N, Melanson L, Lewis E, Santoro N, Pueschel C. 1985. Scanning electron microscopy and infrared spectroscopy of iron sediments formed by *Thiobacillus ferrooxidans*. Geomicrobiol J 4:231–268.

Leathen WW, Kinsel NA, Braley SA Sr. 1956. *Ferrobacillus ferrooxidans*: A chemosynthetic autotrophic bacterium. J Bacteriol 72:700–704.

Lees H, Kwok SC, Suzuki I. 1969. The thermodynamics of iron oxidation by ferrobacilli. Can J Microbiol 15:43–46.

Lieske R. 1919. Zur Ernährungsphysiologie der Eisenbakterien. Z Bakt Parasitenk Infektionskr Hyg Abt II 49:413–425.

Lloyd JR, Blunt-Harris EL, Lovley DR. 1999. The periplasmic 9.6 kDa c-type cytochrome is not an electron shuttle to Fe(III). J Bacteriol 181:7647–7649.

Lobos JH, Chisholm TE, Bopp LH, Holmes DS. 1986. *Acidiphilium organovorum* sp. nov., an acidophilic heterotroph isolated from a *Thiobacillus ferrooxidans* culture. Int J Syst Bacteriol 36:139–144.

Lonergan DJ, Jenter HL, Coates JD, Phillips EJP, Schmidt TM, Lovley DR. 1996. Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J Bacteriol 178:2402–2408.

Lovley DR. 1987. Organic matter mineralization with the reduction of ferric iron: A review. Geomicrobiol J 5:375–399.

Lovley DR. 1991. Dissimilatory Fe(III) and Mn(IV) reductions. Microbiol Rev 55:259–287.

Lovley DR. 2000. Fe(III) and Mn(IV) reduction. In: Lovely DR, ed. Environmental Microbe-Metal Interactions. Washington, DC: ASM Press, pp 3–30.

Lovley DR, Lonergan DJ. 1990. Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron-reducing organism, GS-15. Appl Environ Microbiol 56:1858–1864.

Lovley DR, Phillips EJP. 1986a. Organic matter mineralization with the reduction of ferric iron in anaerobic sediments. Appl Environ Microbiol 51:683–689.

Lovley DR, Phillips EJP. 1986b. Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Appl Environ Microbiol 52:751–757.

Lovley DR, Phillips EJP. 1987. Competitive mechanisms for inhibition of sulfate reduction in sediments. Appl Environ Microbiol 53:2636–2641.

Lovley DR, Phillips EJP. 1988a. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl Environ Microbiol 54:1472–1480.

Lovley DR, Phillips EJP. 1988b. Manganese inhibition of microbial iron reduction in anaerobic sediments. Geomicrobiol J 6:145–155.

Lovley DR, Woodward JC. 1996. Mechanism for chelator stimulation of microbial Fe(III) oxide reduction. Chem Geol 132:19–24.

Lovley DR, Stolz JF, Nord GL, Phillips EJP. 1987. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. Nature (Lond) 330:252–254.

Lovley DR, Phillips EJP, Lonergan DJ. 1989a. Hydrogen and formate oxidation coupled to dissimilatory reduction of iron and manganese by *Alteromonas putrefaciens*. Appl Environ Microbiol 55:700–706.

Lovley DR, Badaeker MJ, Lonergan DJ, Cozzarelli IM, Phillips EJP, Siegel DI. 1989b. Oxidation of aromatic contaminants coupled to microbial iron reduction. Nature (Lond) 339:297–299.

Lovley DR, Phillips EJP, Lonergan DJ. 1991. Enzymatic versus nonenzymatic mechanisms for Fe(III) reduction in aquatic sediments. Environ Sci Technol 25:1062–1967.

Lovley DR, Giovannoni SJ, White DC, Champine JE, Phillips EJP, Gorby YA, Goodwin S. 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch Microbiol 159:336–344.

Lovley DR, Phillips EJP, Lonergan DJ, Widman PK. 1995. Fe(III) and S^0 reduction by *Pelobacter carbinolicus*. Appl Environ Microbiol 61:2132–2138.

Lovley DR, Coates JD, Blunt-Harris EL, Phillips EKP, Woodward JC. 1996. Humic substances as electron acceptors for microbial respiration. Nature (Lond) 382: 445–448.

Lovley DR, Fraga JL, Blunt-Harris EL, Hayes LA, Phillips EJP, Coates JD. 1998. Humic substances as a mediator for microbially catalyzed metal reduction. Acta Hydrochim Hydrobiol 26:152–157.

Lowe KL, DiChristina TJ, Roychoudhury AN, van Cappellen P. 2000. Microbiological and geochemical characterization of microbial Fe(III) reduction in salt marsh sediments. Geomicrobiol J 17:163–178.

Lower SK, Hochella MF Jr, Beveridge TJ. 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and α-FeOOH. Science 292:1360–1363.

Lundgren DG, Dean W. 1979. Biogeochemistry of iron. In: Trudinger PA, Swaine DJ, eds. Biogeochemical Cycling of Mineral-Forming Elements. Amsterdam: Elsevier, pp 211–251.

Lundgren DG, Andersen KJ, Remsen CC, Mahoney RP. 1964. Culture, structure, and physiology of the chemoautotroph *Ferribacillus ferrooxidans*. Dev Indust Microbiol 6:250–259.

Lütters S, Hanert HH. 1989. The ultrastructure of chemolithoautotrophic *Gallionella ferruginea* and *Thiobacillus ferrooxidans* as revealed by chemical fixation and freeze-etching. Arch Microbiol 151:245–251.

Lütters-Czekalla S. 1990. Lithoautotrophic growth of the iron bacterium *Gallionella ferruginea* with thiosulfate or sulfide as energy source. Arch Microbiol 154:417–421.

Lyalikova NN. 1958. A study of chemosynthesis in *Thiobacillus ferrooxidans*. Mikrobiologiya 27:556–559.

Maciag WJ, Lundgren DG. 1964. Carbon dioxide fixation in the chemoautotroph, *Ferrobacillus ferrooxidans*. Biochem Biophys Res Commun 17:603–607.

Mackintosh ME. 1978. Nitrogen fixation by *Thiobacillus ferrooxidans*. J Gen Microbiol 105:215–218.

MacRae IC, Celo JS. 1975. Influence of colloidal iron on the respiration of a species of the genus *Acinetobacter*. Appl Microbiol 29:837–840.

Madsen EL, Morgan MD, Good RE. 1986. Simultaneous photoreduction and microbial oxidation of iron in a stream in the New Jersey pinelands. Limnol Oceanogr 31:832–838.

Magnuson TS, Hodges-Myerson AL, Lovley DR. 2000. Characterization of a membrane-bound NADH-dependent Fe^{3+} reductase from the dissimilatory Fe^{3+}-reducing bacterium *Geobacter sulfurreducens*. FEMS Microbiol Lett 185:205–211.

Mann H, Tazaki T, Fyfe WS, Beveridge TJ, Humphrey R. 1987. Cellular lepidocrocite precipitation and heavy-metal sorption in *Euglena* sp. (unicellular alga): Implications for biomineralization. Chem Geol 63:39–43.

Manning HL. 1975. New medium for isolating iron-oxidizing and heterotrophic acidophilic bacteria from acid mine drainage. Appl Microbiol 30:1010–1016.

Mansch R, Sand W. 1992. Acid-stable cytochromes in ferrous iron oxidizing cell-free preparations from *Thiobacillus ferrooxidans*. FEMS Microbiol Lett 92:83–88.

Mao MWH, Dugan PR, Martin PAW, Tuovinen OH. 1980. Plasmid DNA in chemoorganotrophic *Thiobacilllus ferrooxidans* and *T. acidophilus*. FEMS Microbiol Lett 8:121–125.

Marchlewitz B, Hasche D, Schwartz W. 1961. Untersuchungen über das Verhalten von Thiobakterien gegenüber Schwermetallen. Z Allg Mikrobiol 1:179–191.

Markosyan GE. 1972. A new iron-oxidizing bacterium *Leptospirillum ferrooxidans* gen. et sp. nov. Biol Zh Arm 25:26.

Martin PAW, Dugan PR, Tuovinen OH. 1981. Plasmid DNA in acidophilic, chemolithotrophic thiobacilli. Can J Microbiol 27:850–853.

Mason J, Kelly DP. 1988. Mixotrophic and autotrophic growth of *Thiobacillus acidophilus*. Arch Microbiol 149:317–323.

Mason J, Kelly DP, Wood AP. 1987. Chemolithotrophic and autotrophic growth of *Thermothrix thiopara* and some thiobacilli on thiosulfate and polythionates, and a reassessment of the growth yields of *Thx. thiopara* in chemostat culture. J Gen Microbiol 133:1249–1256.

McClure MA, Wyckoff RWG. 1982. Ultrastructural characteristics of *Sulfolobus acidocaldarius*. J Gen Microbiol 128:433–437.

Merkle FG. 1955. Oxidation-reduction processes in soils. In: Bear FE, ed. *Chemistry of the Soil*. New York: Reinhold, pp 200–218.

Mishra AK, Roy P. 1979. A note on the growth of *Thiobacillus ferrooxidans* on solid medium. J Appl Bacteriol 47:289–292.

Mishra AK, Roy P, Roy Mahapatra SS. 1983. Isolation of *Thiobacillus ferrooxidans* from various habitats and their growth pattern on solid medium. Curr Microbiol 8:147–152.

Molisch H. 1910. Die Eisenbakterien. Jena: Gustav Fischer Verlag.

Munch JC, Ottow JCG. 1977. Modelluntersuchungen zum Mechanismus der bakteriellen Eisenreduktion in Hydromorphen Böden. Z Pflanzenernhr Bodenk 140:549–562.

Munch JC, Ottow JCG. 1980. Preferential reduction of amorphous to crystalline iron oxides by bacteria activity. Soil Sci 129:1–21.

Munch JC, Ottow JCG. 1982. Einfluss von Zellkontakt und Eisen(III)-oxidform auf die bakterielle Eisenreduktion. Z Pflanzenernhr Bodenk 145:66–77.

Munch JC, Hillebrand Th, Ottow JCG. 1978. Transformations in the Fe_o/Fe_d ratio of pedogenic iron oxides affected by iron-reducing bacteria. Can J Soil Sci 58:475–486.

Myers CR, Myers JM. 1992a. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. J Bacteriol 174:3429–3438.

Myers CR, Myers JM. 1992b. Fumarate reductase is a soluble enzyme in anaerobically grown *Shewanella putrefaciens* MR-1. FEMS Microbiol Lett 98:13–20.

Myers CR, Myers JM. 1993. Ferric reductase is associated with the membranes of anaerobically grown *Shewanella putrefaciens* MR-1. FEMS Microbiol Lett 108:15–22.

Myers JM, Myers CR. 2001. Role of outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. Appl Environ Microbiol 67:260–269.

Myers CR, Nealson KH. 1988. Bacterial manganese reduction and growth with manganese oxide as sole electron acceptor. Science 240:1319–1321.

Myers CR, Nealson KH. 1990. Respiration-linked proton translocation coupled to anaerobic reduction of manganese (IV) and iron(III) in *Shewanella putrefaciens* MR-1. J Bacteriol 172:6232–6238.

Nealson KH, Myers CR. 1990. Iron reduction by bacteria: A potential role in the genesis of Banded Iron Formations. Am J Sci 290-A:35–45.

Nealson KH, Saffarini D. 1994. Iron and manganese in anerobic respiration: environmental significance, physiology, and regulation. Annu Rev Microbiol 48:311–343.

Neilands JB, ed. 1974. Microbial Iron Metabolism. New York: Academic Press.

Nevin KP, Lovley DR. 2000. Lack of production of electron-shuttling compounds or solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by *Geobacter metallireducens*. Appl Environ Microbiol 66:2248–2251.

Niemelä SI, Sivalä C, Luoma T, Tuovinen OH. 1994. Maximum temperature limits for acidophilic, mesophilic bacteria in biological leaching systems. Appl Environ Microbiol 60:3444–3446.

Norris PR. 1990. Acidophilic bacteria and their activity in mineral sulfide oxidation. In: Ehrlich HL, Brierley CL, eds. Microbial Mineral Recovery. New York: McGraw-Hill, pp 3–27.

Norris PR. 1997. Thermophiles and bioleaching. In: Rawlings DE, ed. Biomining: Theory, Microbes and Industrial Processes. Berlin: Springer, pp 247–258.

Norris PR, Barr DW. 1985. Growth and iron oxidation by acidophilic moderate thermophiles. FEMS Microbiol Lett 28:221–224.
Norris PR, Brierley JA, Kelly DP. 1980. Physiological characteristics of two facultatively thermophilic mineral-oxidizing bacteria. FEMS Microbiol Lett 7:119–122.
Norris PR, Marsh RM, Lindstrom EB. 1986. Growth of mesophilic and thermophilic bacteria on sulfur and tetrathionate. Biotechnol Appl Biochem 8:313–329.
Norris PR, Barr DW, Hinson D. 1988. Iron and mineral oxidation by acidophilic bacteria: Affinities for iron and attachment to pyrite. In: Norris PR, Kelly DP, eds. *Biohydrometallurgy.* Kew, Surrey, UK: Sci Technol Lett, pp 43–59
Obuekwe CO. 1986. Studies on the physiological reduction of ferric iron by *Pseudomonas sp.* Microbios Lett 33:81–97.
Obuekwe CO, Westlake DWS. 1982. Effect of reducible compounds (potential electron acceptors) on reduction of ferric iron by *Pseudomonas* species. Microbios Lett 19:57–62.
Obuekwe CO, Westlake DWS, Cook FD. 1981. Effect of nitrate on reduction of ferric iron by a bacterium isolated from crude oil. Can J Microbiol 27:692–697.
Ottow JCG. 1969a. The distribution and differentiation of iron-reducing bacteria in gley soils. Zentrabl Bakteriol Parasitenk Infektionskr Hyg Abt 2 123:600–615.
Ottow JCG. 1969b. Der Einfluss von Nitrat, Chlorat, Sulfat, Eisenoxidform und Wachsbedingungen auf das Ausmass der bakteriellen Eisenreduktion. Z Pflanzenernähr Bodenk 124:238–253.
Ottow JCG. 1970a. Selection, characterization and iron-reducing capacity of nitrate reductaseless (nit^-) mutants of iron-reducing bacteria. Z Allg Mikrobiol 10:55–62.
Ottow JCG. 1970b. Bacterial mechanism of gley formation in artificially submerged soil. Nature (Lond) 225:103.
Ottow JCG. 1971. Iron reduction and gley formation by nitrogen-fixing Clostridia. Oecologia 6:164–175.
Ottow JCG, Glathe H. 1971. Isolation and identification of iron-reducing bacteria from gley soils. Soil Biol Biochem 3:43–55.
Ottow JCG, Ottow H. 1970. Gibt es eine Korrelation zwischen der eisenreduzierenden und der nitratreduzierenden Flora des Bodens? Zentrabl Bakteriol Parasitenk Infektionskr Hyg Abt 2 124:314–318.
Ottow JCG, von Klopotek A. 1969. Enzymatic reduction of iron oxide by fungi. Appl Microbiol 18:41–43.
Peng J-B, Yan W-M, Bao XZ. 1994. Expression of heterogenous arsenic resistance genes in the obligately autotrophic biomining bacterium *Thiobacillus ferrooxidans*. Appl Environ Microbiol 60:2653–2656.
Perry EC Jr, Tan FC, Morey GB. 1973. Geology and stable isotope geochemistry of Biwabik Iron Formation, northern Minnesota. Econ Geol 68:1110–1125.
Pichinoty F. 1963. Récherches sur la nitrate réductase d'*Aerobacter aerogens*. Ann Inst Past (Paris) 104:394–418.
Pierson BK, Parenteau MN. 2000. Phototrophs in high iron microbial mats: Microstructure of mats in iron-depositing hot springs. FEMS Microbiol Ecol 32:181–196.
Pierson BK, Parenteau MN, Griffin BM. 1999. Phototrophs in high-iron concentration microbial mats: Physiological ecology of phototrophs in an iron- depositing hot spring. Appl Environ Microbiol 65:5474–5483.

Pivovarova TA, Markosyan GE, Karavaiko GI. 1981. Morphogenesis and fine structure of *Leptospirillum ferrooxidans*. Mikrobiologiya 50:482–486 (Engl transl, pp 339–344).

Pollack JR, Neilands JB. 1970. Enterobactin, an iron transport compound. Biochem Biophys Res Commun 38:989–992.

Praeve P. 1957. Untersuchungen über die Stoffwechselphysiologie des Eisenbakteriums *Leptothrix ochracea* Kützing. Arch Mikrobiol 27:33–62.

Pretorius IM, Rawlings DE, Woods DR. 1986. Identification and cloning of *Thiobacillus ferrooxidans* structural nif-genes in *Escherichia coli*. Gene 45:59–65.

Pretorius IM, Rawlings DE, O'Neill EF, Jones WA, Kirby R, Woods DR. 1987. Nucleotide sequence of the gene encoding the nitrogenase iron protein of *Thiobacillus ferrooxidans*. J Bacteriol 169:367–370.

Pringsheim EG. 1949. The filamentous bacteria *Sphaerotilus*, *Leptothrix* and *Cladothrix* and their relation to iron and manganese. Phil Trans Roy Soc Lond Ser B Biol Sci 233:453–482.

Pronk JT, Johnson DB. 1992. Oxidation and reduction of iron by acidophilic bacteria. Geomicrobiol J 10:149–171.

Pronk JT, Meijer WM, Hazeu W, van Dijken JP, Bos P, Kuenen JG. 1991a. Growth of *Thiobacillus ferrooxidans* on formic acid. Appl Environ Microbiol 57: 2057–2062.

Pronk JT, Liem K, Bos P, Kuenen JG. 1991b. Energy transduction by anaerobic ferric iron respiration in *Thiobacillus ferrooxidans*. Appl Environ Microbiol 57:2063–2068.

Pronk JT, de Bruyn JC, Bos P, Kuenen JG. 1992. Anaerobic growth of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58:2227–2230.

Puchelt H, Schock HH, Schroll E, Hanert H. 1873. Rezente marine Eisenerze aus Santorin, Griechenland. Geol Rundschau 62:786–812.

Rankama K, Sahama TG. 1950. Geochemistry. Chicago: Univ Chicago Press, pp 657–676.

Rawlings DE, Kusano T. 1994. Molecular genetics of *Thiobacillus ferrooxidans*. Microbiol Rev 58:39–55.

Rawlings DE, Pretorius IM, Wodds DR. 1986. Expression of *Thiobacillus ferrooxidans* plasmid function and the development of genetic systems for the thiobacilli. In: Ehrlich HL, Holmes DS, eds. Workshop on Biotechnology for the Mining, Metal-Refining and Fossil Fuel Processing Industrie. Biotech Bioeng Symp 16. New York: Wiley, pp 281–287.

Razzell WE, Trussell PC. 1963. Isolation and properties of an iron-oxidizing *Thiobacillus*. J Bacteriol 85:595–603.

Reid RT, Live DH, Faulkner DJ, Butler A. 1993. A siderophore from a marine bacterium with an exceptional ferric ion affinity constant. Nature (Lond) 366:455–458.

Remsen C, Lundgren DG. 1966. Electron microscopy of the cell envelope of *Ferrobacillus ferrooxidans* prepared by freeze-etching and chemical fixation techniques. J Bacteriol 92:1765–1771.

Roberts JL. 1947. Reduction of ferric hydroxide by strains of *Bacillus polymyxa*. Soil Sci 63:135–140.

Roden EE, Lovley DR. 1993. Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. Appl Environ Microbiol 59:734–742.

Roden EE, Urrutia MM. 1999. Ferrous iron removal promotes microbial reduction of crystalline iron(III) oxides. Environ Sci Technol 33:1847–1853.

Roden EE, Zachara JM. 1996. Microbial reduction of crystalline iron(III) oxides: Influence of oxide surface area and potential for cell growth. Environ Sci Technol 30:1618–1628.

Roden EE, Urrutia MM, Mann CJ. 2000. Bacterial reductive dissolution of crystalline Fe(III) oxide in continuous-flow column reactors. Appl Environ Microbiol 66:1062–1065.

Rosenberg H, Young IG. 1974. Iron transport in the enteric bacteria. In: Neilands JB, ed. Microbial Iron Metabolism: A Comprehensive Treatise. New York: Academic Press, pp 67–82.

Rosselló-Mora RA, Caccavo F Jr, Osterlehner K, Springer N, Spring S, Schüler D, Ludwig W, Amann R, Vanncanney M, Schleifer KH. 1994. Isolation and taxonomic characterization of a halotolerant, facultative iron-reducing bacterium. Syst Appl Microbiol 17:569–573.

Rosselló-Mora RA, Ludwig W, Kämpfer P, Amann R, Schleifer K-H. 1995. *Ferrimonas balearica* gen. nov., spec. nov., a new marine facultative Fe(III)-reducing bacterium. System Appl Microbiol 18:196–202.

Sartory A, Meyer J. 1947. Contribution à l'étude du métabolisme hydrocarboné des bactéries ferrigineuses. CR Acad Sci (Paris) 225:541–542.

Schnaitman CA, Lundgren DG. 1965. Organic compounds in the spent medium of *Ferrobacillus ferrooxidans*. Can J Microbiol 11:23–27.

Schnaitman CA, Korczinski MS, Lundgren DG. 1969. Kinetic studies of iron oxidation by whole cells of *Ferrobacillus ferrooxidans*. J Bacteriol 99:552–557.

Schrader J, Holmes DS. 1988. Phenotypic switching of *Thiobacillus ferrooxidans*. J Bacteriol 170:3915–3923.

Scott DT, McKnoght DM, Blunt-Harris EL, Kolesar SE, Lovley DR. 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Envrion Sci Technol 32:2984–2989.

Seeger M, Jerez CA. 1993. Phosphate starvation-induced changes in *Thiobacillus ferrooxidans*. FEMS Microbiol Lett 108:35–42.

Seeliger S, Cord-Ruwish R, Schink B. 1998. A periplasmic and extracellular c-type cytochrome of *Geobacter sulfurreducens* acts as a ferric iron reductase and as an electron carrier to other acceptors or to partner bacteria. J Bacteriol 180:3686–3691.

Segel IH. 1975. Enzyme Kinetics: Behavior and Analysis or Rapid Equilibrium and Steady-State Enzyme Systems. New York: Wiley.

Segerer AH, Stetter KO. 1998. 29. The Order Sulfolobales. In: Dworkin, M, ed. *The Prokaryotes*. Electronic version. Berlin: Springer-Verlag.

Segerer A, Neuner A, Kristjansson JK, Stetter KO. 1986. *Acidianus infernus*, gen. nov. spec. nov., and *Acidianus brierleyi* comb. nov.: Facultatively aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaebacteria. Arch Microbiol 36:559–564.

Selkov E, Overbeek R, Kogan Y, Chu L, Vonstein V, Holmes D, Silver S, Haselkorn R, Fonstein M. 2000. Proc Natl Acad Sci (USA) 97:3509–3514.

Shafia F, Wilkinson RF. 1969. Growth of *Ferrobacillus ferrooxidans* on organic matter. J Bacteriol 97:256–260.

Shafia F, Brinson KR, Heinzman MW, Brady JM. 1972. Transition of chemolithotroph *Ferrobacilllus ferrooxidans* to obligate organotrophy and metabolic capabilities of glucose-grown cells. J Bacteriol 111:56–65.

Short KA, Blakemore RP. 1986. Iron respiration-driven proton translocation in aerobic bacteria. J Bacteriol 167:729–731.

Silver M, Ehrlich HL, Ivarson KC. 1986. Soil mineral transformation mediated by soil microbes. In: Huang PM, Schnitzer M, eds. Interaction of Soil Minerals with Natural Organics and Microbes. SSSA Spec Publ 17. Madison, WI: Soil Sci Soc Am, pp 497–519.

Silverman MP, Lundgren DG. 1959a. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. J Bacteriol 59:642–647.

Silverman MP, Lundgren DG. 1959b. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. II. Manometric studies. J Bacteriol 78:326–331.

Sørensen J. 1987. Nitrate reduction in marine sediment: Pathways and interactions with iron and sulfur cycling. Geomicrobiol J 5:401–421.

Sørensen J, Jørgensen BB. 1987. Early diagenesis in sediments from Danish coastal waters: Microbial activity and Mn-Fe-S geochemistry. Geochim Cosmochim Acta 51:1583–1590.

Starkey RL. 1945. Precipitation of ferric hydrate by iron bacteria. Science 102:532–533.

Starkey RL, Halvorson HO. 1927. Studies on the transformations of iron in nature. II. Concerning the importance of microorganisms in the solution and precipitation of iron. Soil Sci 24:381–402.

Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, eds. 1981. The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Berlin: Springer-Verlag.

Stevens CJ, Dugan PR, Tuovinen OH. 1986. Acetylene reduction (nitrogen fixation) by *Thiobacillus ferrooxidans*. Biotechnol Appl Biochem 8:351–359.

Stokes JL. 1954. Studies on the filamentous sheathed iron bacterium *Sphaerotilus natans*. J Bacteriol 67:278–291.

Straub KL, Benz M, Schink B, Widdel F. 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl Environ Microbiol 62:1458–1460.

Stucki JW, Roth CB. 1977. Oxidation-reduction mechanism for structural iron in nontronite. Soil Sci Soc Am J 41:808–814.

Stucki JW, Golden DC, Roth CB. 1984. Effects of reduction and reoxidation of structural iron on the surface charge and dissolution of dioctahedral smectites. Clays Clay Min 32:350–356.

Stucki JW, Komadel P, Wilkinson, HT. 1987. Microbial reduction of structural iron(III) in smectites. Soil Sci Soc Am J 51:1663–1665.

Stucki JW, Wu J, Gan H, Komadel P, Banin A. 2000. Effects of iron oxidation state and organic cations on dioctahedral smectite hydration. Clays Clay Min 48:290–298.

Sugio T, Anzai Y, Tano T, Imai K. 1981. Isolation and some properties of an obligate and a facultative iron-oxidizing bacterium. Agric Biol Chem 45:1141–1151.

Sugio T, Domatsu C, Munakata O, Tano T, Imai K. 1985. Role of a ferric iron reducing system in sulfur oxidation by *Thiobacillus ferrooxidans*. Appl Environ Microbiol 49:1401–1406.

Sugio T, Wada K, Mori M, Inagaki K, Tano T. 1988a. Synthesis of an iron-oxidizing system during growth of *Thiobacillus ferrooxidans* on sulfur-basal salts medium. Appl Environ Microbiol 54:150–152.

Sugio T, Katagiri T, Moriyama M, Zhen YL, Inagaki K, Tano T. 1988b. Existence of a new type of sulfite oxidase which utilizes ferric ions as an electron acceptor in *Thiobacillus ferrooxidans*. Appl Environ Microbiol 54:153–157.

Sugio T, Katagiri T, Inagaki K, Tano T. 1989. Actual substrate for elemental sulfur oxidation by sulfur : ferric ion oxidoreductase purified from *Thiobacilllus ferrooxidans*. Biochim Biophys Acta 973:250–256.

Sugio T, Hirose T, Lii-Zhen Y, Tano T. 1992a. Purification and some properties of sulfite : ferric ion oxidoreductase from *Thiobacillus ferrooxidans*. J Bacteriol 174:4189–4192.

Sugio T, White KJ, Shute E, Choate D, Blake RC II. 1992b. Existence of a hydrogen sulfide : ferric ion oxidoreductase in iron-oxidizing bacteria. Appl Environ Microbiol 58:431–433.

Tabita R, Lundgren DG. 1971a. Utilization of glucose and the effect of organic compounds on the chemolithotroph *Thiobacillus ferrooxidans*. J Bacteriol 108:328–333.

Tabita R, Lundgren DG. 1971b. Heterotrophic metabolism of the chemolithotroph *Thiobacillus ferrooxidans*. J Bacteriol 108:334–342.

Tait GH. 1975. The identification and biosynthesis of siderochromes formed by *Micrococcus denitrificans*. Biochem J 146:191–204.

Takai Y, Takamura T. 1966. The mechanism of reduction in water-logged paddy soil. Folia Microbiol 11:304–315.

Taylor KA, Deatherage JF, Amos LA. 1982. Structure of the S-layer of *Sulfolobus acidocaldarius*. Nature (Lond) 299:840–842.

Temple KL, Colmer AR. 1951. The autotrophic oxidation of iron by a new bacterium: *Thiobacillus ferrooxidans*. J Bacteriol 62:605–611.

Thamdrup B, Finster K, Wuergler Hansen J, Bak F. 1993. Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. Appl Environ Microbiol 99:101–108.

Tikhonova GV, Lisenkova LL, Doman NG, Skulachev VP. 1967. Electron transport pathways in *Thiobacillus ferrooxidans*. Mikrobiologiya 32:725–734 (Engl transl, pp 599–606.

Tipping E, Thompson DW, Ohnstad M, Hetherington NB. 1986. Effects of pH on the release of metals from naturally occurring oxides of manganese and iron. Environ Technol Lett 7:109–114.

Trafford BD, Bloomfield C, Kelso WI, Pruden G. 1973. Ochre formation in field drains in pyritic soils. J Soil Sci 24:453–460.

Troshanov EP. 1968. Iron- and manganese-reducing microorganisms in ore-containing lakes of the Karelian isthmus. Mikrobiologiya 37:934–940 (Engl transl, pp 786–791).

Troshanov EP. 1969. Conditions affecting the reduction of iron and manganese by bacteria in the ore-bearing lakes of the Karelian isthmus. Mikrobiologiya 38:634–643 (Engl transl, pp 528–535).

Tuovinen OH, Kelly DP. 1973. Studies on the growth of *Thiobacillus ferrooxidans*. I. Use of membrane filters and ferrous iron agar to determine viable numbers, and comparison with $^{14}CO_2$-fixation and iron oxidation as measures of growth. Arch Mikrobiol 88:285–298.

Tuovinen OH, Kelly DP. 1974. Studies on the growth of *Thiobacilllus ferrooxidans*. II. Toxicity of uranium to growing cultures and tolerance conferred by mutation, other metal cations and EDTA. Arch Microbiol 95:153–164.

Tuovinen OH, Nicholas DJD. 1977. Transition of *Thiobacillus ferrooxidans* KG-4 from heterotrophic growth on glucose to autotrophic growth on ferrous-iron. Arch Microbiol 114:193–195.

Tuovinen OH, Niemal SI, Gyllenberg HG. 1971. Tolerance of *Thiobacillus ferrooxidans* to some metals. Antonie v Leeuwenhoek 37:489–496.

Tuttle JH, Dugan PR. 1976. Inhibition of growth, iron, and sulfur oxidation by *Thiobacillus ferrooxidans* by simple organic compounds. Can J Microbiol 22: 719–730.

Unz RF, Lundgren DG. 1961. A comparative nutritional study of three chemoautotrophic bacteria: *Ferrobacillus ferrooxidans*, *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Soil Sci 92:302–313.

Vartanian SE, Cowart RE. 1999. Extracellular iron reductases: Identification of a new class of enzymes by siderophore-producing microorganisms. Arch Biochem Biophys 364:75–82.

Venkateswaran K, Moser DP, Dollhopf ME, Lies DP, Saffariui DA, McGregor BJ, Ringelberg DB, White DC, Nishijima M, Sano H, Burghardt J, Stackebrandt E, Nealson KH. 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. Int J Syst Bacteriol 49(2):705–724.

Vernon LP, Mangum JH, Beck JV, Shafia FM. 1960. Studies on a ferrous-ion-oxidizing bacterium. II. Cytochrome composition. Arch Biochem Biophys 88:227–231.

Vorreiter L, Madgwick JC. 1982. The effect of sodium chloride on bacterial leaching of low-grade copper ore. Proc Australas Inst Min Metall 284:63–66.

Walker JCG. 1987. Was the Archean biosphere upside down? Nature (Lond) 329:710–712.

Walker JCG, Klein C, Schidlowski M, Schopf JW, Stevenson DJ, Walter MR. 1983. Environmental evolution of the Archean-Early Proterozoic Earth. In: Schopf JW, ed. Earth's Earliest Biosphere: Its Origin and Evolution. Princeton, NJ: Princeton Univ Press, pp 260–290.

Walsh F, Mitchell R. 1972. An acid-tolerant iron-oxidizing *Metallogenium*. J Gen Microbiol 72:369–376.

Wichlacz PL, Unz RF, Langworthy TA. 1986. *Acidiphilium angustum* sp. nov., *Acidiphilium facilis* sp. nov., and *Acidiphilium rubrum* sp. nov.: Acidophilic heterotrophic bacteria isolated from acid coal mine drainage. Int J Syst Bacteriol 36:197–201.

Widdel F, Schnell S, Heising S, Ehrenreich A, Assmus B, Schink B. 1993. Ferrous oxidation by anoxygenic phototrophic bacteria. Nature (Lond) 362:834–836.

Williams HD, Poole RK. 1987. Reduction of iron(III) by *Escherichia coli* K12: Lack of involvement of the respiratory chains. Curr Microbiol 15:319–324.
Winogradsky S. 1888. über Eisenbakterien. Bot Ztg 46:261–276.
Winogradsky S. 1922. Eisenbakterien als Anorgoxydanten. Zentrabl Baketeriol Parasitenk Infektionskr Hyg Abt II 57:1–21.
Wolfe RS. 1964. Iron and manganese bacteria. In: Heukelakian H, Dondero N, eds. Principles and Applications in Aquatic Microbiology. New York: Wiley, pp 82–97.
Yamanaka T, Yano T, Kai M, Tamegai H, Sato A, Fukumori Y. 1991. The electron transfer system in an acidophilic iron-oxidizing bacterium. In: Mukohata Y, ed. *New Era of Bioenergetics*. Tokyo: Academic Press, pp 223–246.
Yates JR, Holmes DS. 1987. Two families of repeated DNA sequences in *Thiobacillus ferrooxidans*. J Bacteriol 169:1861–1870.
Yates MG, Nason A. 1966a. Enhancing effect of nucleic acids and their derivatives in the reduction of cytochrome c by ferrous ions. J Biol Chem 241:4861–4871.
Yates MG, Nason A. 1966b. Electron transport systems of the chemoautotroph *Ferrobacillus ferrooxidans*. II. Purification and properties of a heat-labile iron-cytochrome c reductase. J Biol Chem 241:4872–4880.
Zavarzin GA. 1972. Heterotrophic satellite of *Thiobacillus ferrooxidans*. Mikrobiologiya 41:369–370 (Engl transl, pp 323–324).

16

Geomicrobiology of Manganese

16.1 OCCURRENCE OF MANGANESE IN THE EARTH'S CRUST

The abundance of manganese in the Earth's crust has been estimated to be 0.1% (Alexandrov, 1972, p. 670). The element is, therefore, only 1/50 as plentiful as iron in this part of the Earth. Its distribution in the crust is by no means uniform. In soils, for instance, its concentration can range from 0.002% to 10% (Goldschmidt, 1954). An average concentration in freshwater has been reported to be $8\,\mu g\,kg^{-1}$ (Bowen, 1979). Concentrations slightly in excess of $1\,mg\,kg^{-1}$ can be encountered in anoxic hypolimnia of some lakes. In seawater, an average concentration has been reported to be $0.2\,\mu g\,kg^{-1}$ (Bowen, 1979), but concentrations more than three orders of magnitude greater can be encountered near active hydrothermal vents on mid-ocean spreading centers.

Manganese is found as a major or minor component in more than 100 naturally occurring minerals (Bureau of Mines, 1965, p. 556). Major accumulations of manganese occur in the form of oxides, carbonates, and silicates. Among the oxides, psilomelane [$Ba,Mn^{2+}, (Mn^{4+})_8O_{16}(OH)_4$], birnessite ($\partial MnO_2$), pyrolusite and vernadite (MnO_2), manganite (MnOOH), hausmannite (Mn_3O_4), and todorokite [$(Mn^{2+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, K^+, Na^+)_2(Mn^{4+})_5O_{12}\cdot 3H_2O$] are important examples. Among the carbonates, rhodochrosite ($MnCO_3$) is important, and among the silicates, rhodonite ($MnSiO_3$) and braunite [$Mn,Si)_2O_3$] are

important. The oxides, carbonates, and silicates of manganese originated mostly as secondary authigenic minerals from the precipitation of dissolved manganese. Minerals that contain manganese as a minor constituent include ferromagnesian minerals such as pyroxenes and amphiboles (Trost, 1958) or micas such as biotite (Lawton, 1955, p. 59). These are all of igneous origin.

16.2 GEOCHEMICALLY IMPORTANT PROPERTIES OF MANGANESE

Manganese is one of the elements of the first transition series in the periodic table, which includes, in order of increasing atomic number from 21 to 29, the elements Sc, Ti, V, Cr, Mn, Fe, Co, Ni, and Cu. Electronically, these elements differ mostly in the degree to which their *d* orbitals are filled (Drew, 1972). Their increasing oxidation states are attributed to removal of 4*s* and 3*d* electrons (Sienko and Plane, 1966).

Manganese can exist in the oxidation states 0, +2, +3, +4, +6, and +7. However, in nature only the +2, +3, and +4 oxidation states are commonly found. Of the three naturally occurring oxidation states, only manganese in the +2 oxidation state can occur as a free ion in aqueous solution. In this oxidation state it may also occur as a soluble inorganic or organic complex. Manganese in the +3 oxidation state can occur in aqueous solution only when it is complexed. The free +3 ion tends to disproportionate (dismutate) into the +2 and +4 oxidation states:

$$2Mn^{3+} + 2H_2O \Leftrightarrow Mn^{2+} + MnO_2 + 4H^+ \tag{16.1}$$

The +4 oxides of manganese are insoluble in water. They have amphoteric properties that account for their affinity for various cations, especially for heavy metal ions such as Co^{2+}, Ni^{2+}, and Cu^{2+}. Mn(IV) oxides have long been known as scavengers of metallic cations (Geloso, 1927; Goldberg, 1954). They are frequently associated with ferric iron in nature.

Manganous ion is more stable than ferrous ion under similar conditions of pH and E_h. Based on equilibrium computations, manganese should exist predominantly as Mn^{2+} below pH 5.5 and 3.8×10^{-4} atm of CO_2, and as Mn(IV) above pH 5.5 if the E_h is approximately 800 mV and the Mn^{2+} activity is 0.1 ppm. At an E_h of 500 mV and below, Mn^{2+} at an activity as high as 10 ppm may predominate up to pH 7.8–8.0 (Hem, 1963). Although in theory 0.1 ppm of Mn^{2+} ions in aqueous solution should readily autoxidize when exposed to air at pH values above 4, they usually do not do so until the pH exceeds 8. Apart from Mn^{2+} concentration and E_h effects, one explanation for this resistance of Mn^{2+} ions to oxidation is the high energy of activation requirement for the reaction (Crerar and Barnes, 1974). Another explanation is that the Mn^{2+} may be

extensively complexed and thereby stabilized by such inorganic ions as Cl^-, SO_4^{2-}, and HCO_3^- (Hem, 1963; Goldberg and Arrhenius, 1958) or by organic compounds such as amino acids, humic acids, and others (Graham, 1959; Hood, 1963; Hood and Slowey, 1964).

16.3 BIOLOGICAL IMPORTANCE OF MANGANESE

Manganese is an important trace element in biological systems. It is essential in microbial, plant, and animal nutrition. It is required as an activator by a number of enzymes such as isocitric dehydrogenase or malic enzyme and may replace Mg^{2+} ion as an activator, for example in enolase (Mahler and Cordes, 1966). It is also required in oxygenic photosynthesis, where it functions in the production of oxygen from water by photosystem II (e.g., Klimov, 1984). In Section 16.5, the ability of Mn(II) to serve as an energy source for some bacteria will be discussed, and in Section 16.6, the ability of Mn(III) and Mn(IV) to serve as terminal electron acceptors in respiration by some bacteria will be examined. As in the case of iron, the most important geomicrobial interactions with manganese are those that lead to precipitation of dissolved manganese in an insoluble phase (immobilization) or solubilization of insoluble forms of manganese (mobilization). These reactions frequently but not always involve oxidations or reductions of manganese.

16.4 MANGANESE-OXIDIZING AND -REDUCING BACTERIA AND FUNGI

Manganese-Oxidizing Bacteria and Fungi

Jackson (1901a, 1901b) and Beijerinck (1913) were the first to describe the existence of manganese-oxidizing bacteria. Since their discovery, a significant number of different kinds of bacteria, many of which are taxonomically unrelated, have been reported to oxidize manganese. Among these, some promote the oxidation enzymatically, others nonenzymatically. With still other bacteria, it is as yet unclear whether they oxidize manganese enzymatically or nonenzymatically. To date, all known bacterial manganese oxidizers seem to belong to the domain Bacteria; none have so far been found that belong to the domain Archaea. They include gram-positive and gram-negative forms and are represented by spore-forming and non-spore-forming rods, cocci, vibrios, spirilla, and sheathed and appendaged bacteria. No bona fide autotrophic manganese oxidizers have so far been identified, although two unconfirmed claims of autotrophy have appeared in the bacteriological literature (Ali and Stokes, 1971; Kepkay and Nealson, 1987). Caspi et al. (1996) reported finding a cryptic ribulose 1,5-bisphosphate carboxylase/oxygenase gene in a marine, gram-negative α-proteobacterium strain S185-

9A1, which did not couple CO_2 fixation to Mn^{2+} oxidation. In most instances, growth in the presence of manganese is either mixotrophic (manganese oxidation supplies some or all of the energy needed by the organism, but its carbon source is organic) or heterotrophic (manganese oxidation furnishes no useful energy; carbon and energy are derived from organic carbon). Table 16.1 lists examples of bacteria that have been shown to oxidize manganese enzymatically or nonenzymatically. They have been detected in very diverse environments, such as "desert varnish" on rock surfaces, in soil, in the water column and sediments of freshwater lakes and streams, and in ocean water and sediments, including on and in ferromanganese concretions on the ocean floor.

Some mycelium-forming fungi have also been found to promote manganese oxidation, at least under laboratory conditions. In most instances, this oxidation is probably nonenzymatic and due to interaction with a metabolic product (e.g., hydroxy acid) or a fungal cell component. However, in the case of some fungi, such as the white rot fungus *Phanerochaete chrysosporium*, oxidation may be the result of an extracellular Mn(II)-dependent peroxidase that oxidizes Mn(II) to Mn(III) with the consumption of H_2O_2 (Glenn and Gold, 1985; Glenn et al., 1986). This reaction is similar to one observed with peroxidase in plant extracts (horseradish, turnip) (Kenten and Mann, 1949, 1950). The Mn(III) is stabilized as a complex $\{Mn^{3+}\}$, e.g., lactate complex, which may then react with an organic compound (YH) such as a lignin component that reduces the Mn(III) back to Mn(II) and is itself oxidized (Fig. 16.1) (Glenn et al., 1986; Paszczynski et al., 1986). This re-reduction of Mn(III) to Mn(II) makes manganese merely an electron shuttle in the peroxidase system and has no geochemical significance insofar as manganese redistribution is concerned. However, Kenten and Mann (1949) proposed that at very low H_2O_2 concentration, oxidized manganese may accumulate because reduction of the oxidized manganese would be negligible. In the peroxidase M2 reaction in the absence of YH, Glenn et al. (1986) observed the formation of a brown precipitate that was a manganese oxide (tentatively identified as MnO_2, but could have been Mn_2O_3 or Mn_3O_4) in a laboratory experiment in which H_2O_2 slowly diffused into a weakly buffered solution of enzyme and Mn(II).

Höfer and Schlosser (1999) found a laccase produced by the fungus *Trametes versicolor* that in pure culture oxidized Mn^{2+} to Mn^{3+} in the presence of pyrophosphate. O_2 was the terminal electron acceptor and not H_2O_2, as in the case of manganese peroxidase. The enzyme is one of the multicopper oxidases.

Manganese-Reducing Bacteria and Fungi

A number of different taxonomically unrelated bacteria have been found to reduce manganese either enzymatically or nonenzymatically. The bacteria that reduce manganese enzymatically often do so as a form of respiration in which

TABLE 16.1 Some Bacteria That Oxidize Manganese

A. Attack dissolved Mn^{2+} enzymatically
   1. Derive useful energy:
      Marine strains SSW_{22}, S_{13}, HCM-41, and E_{13} (all are gram-negative rods) (Ref. 1; unpublished results)
      Hyphomicrobium manganoxidans (Ref. 2)
      Pseudomonas strain S-36 (Ref. 3)
   2. Do not derive useful energy:
      Arthrobacter siderocapsulatus (Ref. 4)
      Leptothrix discophora (Ref. 17)
      Leptothrix pseudoochracea (Ref. 4)
      Metallogenium (Ref. 4)
      Strain FMn-1 (Ref. 5)
   3. Not known if able to derive useful energy:
      Aeromonas sp. (Nealson, 1978)
      Arthrobacter B (Ref. 6)
      Arthrobacter citreus (Ref. 7)
      Arthrobacter globiformis (Ref. 7)
      Arthrobacter simplex (Ref. 7)
      Citrobacter freundii E_4 (Ref. 8)
      Flavobacterium (Ref. 19)
      Hyphomicrobium T37 (Ref. 9)
      Pedomicrobium (Ref. 10)
      Pseudomonas E_1 (Ref. 8)
      Pseudomonas putida GB-1 (Ref. 20)
      Pseudomonas putida Mn-1 (Ref. 20)
      Pseudomonas spp. (Refs. 11, 19)

B. Attack Mn^{2+} prebound to Mn(IV) oxide or some clays enzymatically
   1. Derive useful energy:
      Arthrobacter 37 (Ref. 12)
      Oceanospirillum (Ref. 13)
      Marine strain CFP-11 (Ref. 1)

C. Attack Mn^{2+} nonenzymatically
   Pseudomonas manganoxidans (Ref. 14; but see also Ref. 20)
   Streptomyces sp. (Ref. 15)
   Bacillus SG-1 (Ref. 16; but see also Ref. 18)

Source: (1) Ehrlich, 1983, 1985; Ehrlich and Salerno, 1990; (2) Eleftheriadis, 1976; (3) Kepkay and Nealson, 1987; (4) Dubinina, 1978a, 1978b; (5) Zindulis and Ehrlich, 1983; (6) Bromfield, 1956; Bromfield and David, 1976; (7) Dubinina and Zhdanov, 1975; (8) Douka, 1977; (9) Tyler and Marshall, 1967b; (10) Aristovskaya, 1961; Ghiorse and Hirsch, 1979; (11) Zavarzin, 1962; (12) Ehrlich, 1968; Arcuri, 1978; (13) Ehrlich 1976, 1978a; (14) Jung and Schweisfurth, 1979; (15) Bromfield, 1978, 1979; (16) de Vrind et al., 1986b; (17) Adams and Ghiorse, 1987; (18) Van Wassbergen et al., 1993; (19) Nealson, 1978; (20) Tebo et al., 1997.

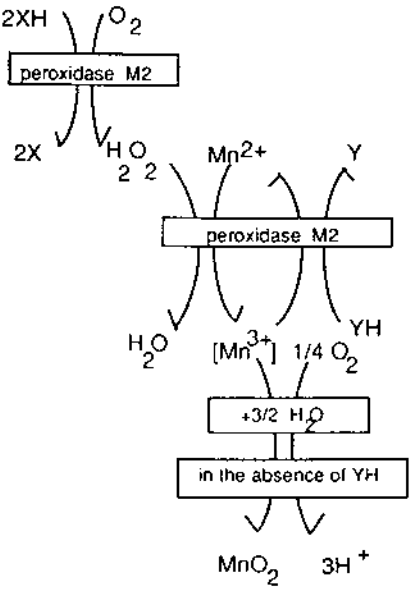


FIG. 16.1 Interactions of extracellular peroxidase M2 from *Phanerochaete chrysosporium* with manganese. (Based on description by Glenn et al., 1986, Pasczynski et al., 1986.)

oxidized manganese serves as terminal electron acceptor and is reduced to Mn(II). Some bacteria can reduce the oxidized manganese aerobically or anaerobically, whereas others can reduce it only anaerobically. Thus, manganese-reducing bacteria include aerobes and strict and facultative anaerobes. In some cases, manganese may be reduced to satisfy a nutritional need for soluble Mn(II) (see de Vrind et al., 1986a; also discussion by Ehrlich, 1987) or to scavenge excess reducing power, as in some cases of NO_3^- reduction (Robertson et al., 1988) and ferric reduction (Ghiorse, 1988; Lovley, 1991).

Bacteria that reduce manganese oxides include gram-positive and gram-negative forms, including rods, cocci, and vibrios, all belonging to the domain Bacteria. The majority of the bacteria studied to date that can respire with manganese(IV) oxide as terminal electron acceptor use reduced carbon as electron donor (reductant), but a few types can also use H_2 anaerobically. Like manganese oxidizers, they have been found in very diverse environments, including soil and deep subsurface, freshwater, and marine habitats. Most of the manganese-reducing bacteria described to date do not seem to have the ability to oxidize Mn(II) as well, but a few exceptions have been reported. Representative examples of Mn reducers are listed in Table 16.2.

TABLE 16.2 Some Mn(IV)-Reducing Bacteria

A. Gram-positive bacteria
Bacillus 29 (Ref. 1)
Coccus 32 (Ref. 1)
Bacillus SG-1 (Ref. 2)
Bacillus circulans (Ref. 3)
Bacillus polymyxa (Ref. 3)
Bacillus mesentericus (Ref. 3)
Bacillus mycoides (Ref. 3)
Bacillus cereus (Ref. 3)
Bacillus centrosporus (Ref. 3)
Bacillus filaris (Ref. 3)
Arthrobacter strain B (Ref. 4)
Bacillus GJ33 (Ref. 5)
B. Gram-negative bacteria
Geobacter metallireducens (formerly strain GS-15) (Ref. 6)
Geobacter sulfurreducens (Ref. 7)
Shewanella (formerly *Alteromonas*) *putrefaciens* (Ref. 8)
Pseudomonas liquefaciens (Ref. 3)
Strain BIII 32 (Ref. 9)
Strain BIII 41 (Ref. 9)
Strain BIII 88 (Ref. 11)
Acinetobacter calcoaceticus (Ref. 10)

Source: (1) Trimble and Ehrlich, 1968; (2) de Vrind et al., 1986a; (3) Troshanov, 1968, 1969; (4) Bromfield and David, 1976; (5) Ehrlich et al., 1973; (6) Lovley and Phillips, 1988a; (7) Caccavo et al., 1994; (8) Myers and Nealson, 1988a; (9) Ehrlich, 1980; (10) Karavaiko et al., 1986; (11) Ehrlich, 1993a.

Some mycelial fungi have been found to reduce manganese oxides, at least under laboratory conditions. As in the case of Mn(II) oxidation by fungi, reduction of manganese oxides by them must be nonenzymatic in most cases, although experimental proof for any specific mechanism is mostly lacking. Nonenzymatic reduction of manganese oxides by fungi is best explained in terms of production of metabolic products by them, which act as reductants.

16.5 BIO-OXIDATION OF MANGANESE

Like iron oxidation, manganese oxidation by microbes may be enzymatic or nonenzymatic. However, unlike enzymatic iron oxidation, it has not been reported to occur at very acid pH. Enzymatic iron oxidation in air-saturated solutions or solutions close to air saturation is favored by very acid pH because at

that pH iron does not autoxidize at a significant rate. Enzymatic manganese oxidation in solution under similar air saturation levels is not favored at pH values much lower than neutrality. This is because, in contrast to iron, the standard free energy change (ΔG^0) when manganese is oxidized with oxygen as electron acceptor decreases steadily until it assumes a positive value near pH 1.0 (see standard free energy values at pH 0 and 7 listed by Ehrlich, 1978a). One instance of nonenzymatic manganese bio-oxidation at a pH of around 5.0 has been documented (Bromfield, 1978, 1979).

Among the first to report on bacterial manganese oxidation were Jackson (1901a, 1901b) and Beijerinck (1913). The latter suggested that it may be associated with autotrophic growth. Lieske (1919) and Sartory and Meyer (1947) suggested that manganese oxidation may also be associated with mixotrophic growth. Either way, an enzymatic process was implied. The first experiments to demonstrate Mn^{2+} oxidation by resting (nongrowing) cells were performed by Bromfield (1956). He showed that stationary growth phase cells of *Arthrobacter* strain B (formerly called *Corynebacterium* strain B) oxidized Mn^{2+} in a 0.005% $MnSO_4 \cdot 4H_2O$ solution at 40°C but not above 45°C. The oxidation was inhibited by copper and mercury salts and by azide and cyanide. In a later study, Bromfield (1974) showed that the composition of organic substrate and its concentration affected manganese oxidation by *Arthrobacter* strain B. Bromfield and David (1976) reported that cells of *Arthrobacter* strain B rapidly adsorbed Mn^{2+} ions from solution as well as oxidizing them. The adsorbed but unoxidized Mn^{2+} could be displaced by the addition of 5 mM Cu^{2+}. Manganese oxidation kinetics of this organism are shown in Figure 16.2. Bromfield and David (1976) also found that this organism could reduce oxidized manganese. Most studies to date leave the impression that enzymatic manganese oxidation is restricted mainly to bacteria. An exceptional case of manganese oxidation by a fungus is discussed in Section 16.4.

Enzymatic Manganese Oxidation

On a physiological basis, various reports of enzymatic manganese(II) oxidation by bacteria suggests that not all of them use the same mechanism. However, on a molecular basis, some degree of commonality among four cultures—two belonging to the genus *Pseudomonas*, one to *Leptothrix*, and one to *Bacillus*—may exist. They all seem to employ a multicopper oxidase in the oxidation (Brouwers et al., 2000a). Another common feature of these cultures is that they deposit the oxidized manganese on their cells, sheath, or spore, respectively (Okazaki et al., 1997; Mulder, 1972; Rosson and Nealson, 1982). Although these molecular studies have led some to suggest that this oxidase is common to all manganese oxidizers, physiological considerations of various other manganese-oxidizing bacteria described in the literature raise doubt about a universal manganese-oxidizing mechanism. In what follows, a *physiological classification* is presented

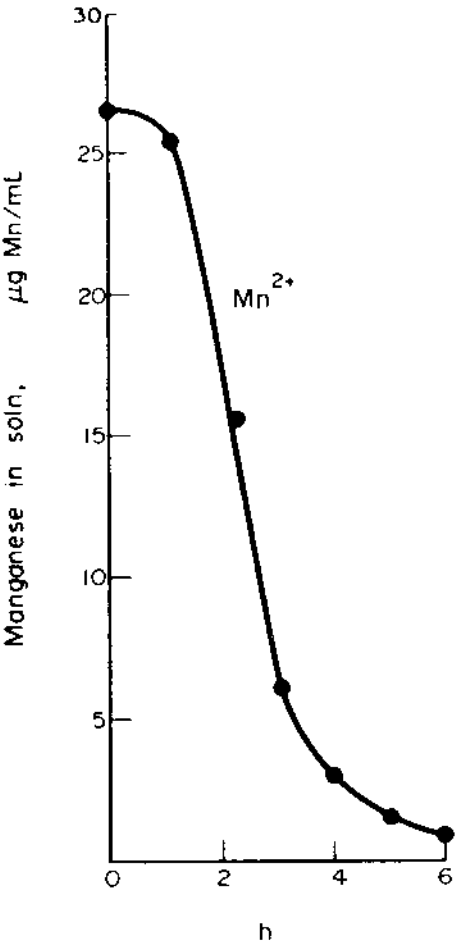


FIG. 16.2 Oxidation of 0.5 mM Mn^{2+} by bacterial cell suspension of *Arthrobacter* strain B (formerly *Corynebacterium* strain B) at pH 7.0. The mixture (80 mL) contained 4 mg cells per milliliter and 27 µg of Mn per milliliter. (From *Soil Biology and Biochemistry.* Bromfield SM and David DJ. Copyright 1976, Pergamon Press, with permission.)

based on what is known about these bacteria. For the majority of bacteria a molecular analysis is lacking. The classification includes three major groups, at least one of which can be further divided into several subgroups (Table 16.3).

Group I Manganese Oxidizers

Group I manganese oxidizers include those bacteria that oxidize dissolved Mn(II) species, e.g., Mn^{2+}, using oxygen as terminal electron acceptor. Some bacteria in

TABLE 16.3 Enzymatic Mechanisms in Mn-Oxidizing Bacteria

Group I manganese-oxidizers oxidize dissolved manganese (Mn^{2+}) with O_2 as terminal electron acceptor

Subgroup Ia: Conserve energy from Mn^{2+} oxidation

Subgroup Ib: Do not conserve energy from Mn^{2+} oxidation. They use oxidized soluble factor in periplasm as oxidant of Mn^{2+}. The resultant reduced factor is reoxidized in cytoplasm and returned to periplasm.

Subgroup Ic: Do not conserve energy from Mn^{2+} oxidation. This subgroup includes some sheathed bacteria and spores of *Bacillus* SG-1 and a strain of *B. megaterium*.

Group II manganese-oxidizers oxidize Mn^{2+} with O_2 as terminal electron acceptor when the Mn^{2+} is prebound to a suitable binding agent such as Mn(IV) oxide, ferromanganese, montmorillonite, or kaolinite. Group II oxidizers conserve energy from the oxidation.

Group III manganese-oxidizers oxidize Mn^{2+} with H_2O_2 as oxidant catalyzed by catalase.

this group can gain useful energy from this reaction (Subgroup 1a), whereas others do not (Subgroup 1b). The overall oxidation reaction can be summarized as follows:

$$Mn^{2+} + 0.5O_2 + H_2O \rightarrow MnO_2 + 2H^+ \tag{16.2}$$

Some types of bacteria in Group I whose manganese-oxidizing system has been examined to date appear to oxidize manganese in their cell envelope external to the plasma membrane, i.e., in the periplasm when gram-negative bacteria are involved, or the wall region (periplasmic equivalent: see Hobot et al., 1984; Beverige and Kadurugamuwa, 1996) when gram-positive bacteria are involved (Ehrlich, 1983; Ehrlich and Zapkin, 1985). In *Pseudomonas putida* strains MnB1 and GB-1, both gram-negative, the manganese oxidase, a protein complex that includes a multicopper oxidase, appears to reside in the outer membrane and Mn^{2+} oxidation occurs at the cell surface (Okazaki et al., 1997; Brouwers et al., 2000a).

Subgroup Ia. In bacteria of Subgroup Ia, Mn^{2+} oxidation can be coupled to ATP synthesis (energy conservation), as has been demonstrated directly with everted membrane vesicles from a marine bacterium, strain SSW_{22} (Ehrlich, 1983; Ehrlich and Salerno, 1990) or by uncoupling of ATP synthesis in this organism with 2,4-dinitrophenol (Ehrlich and Salerno, 1988, 1990). Synthesis of ATP in strain SSW_{22} appears to be very tightly coupled to manganese oxidation,

because added ADP stimulates manganese oxidation by everted membrane vesicles from the organism (Ehrlich and Salerno, 1990). A periplasmic factor is required for Mn^{2+} oxidation by membranes from strain SSW_{22} (Clark and Ehrlich, 1988; Clark, 1991), but the exact function of this factor (e.g., electron transfer or chelation of Mn^{2+}) in the system has yet to be established. A diagram illustrating the current working model to explain coupling of ATP synthesis to manganese oxidation in Group Ia bacteria is shown in Figure 16.3.

The model assumes that chemiosmosis is the underlying energy-transducing mechanism. The manganese-oxidizing half-reaction occurs external to the plasma membrane in the periplasm or its equivalent. It also assumes that oxygen reduction occurs on the inner surface of the plasma membrane. Proton generation by the manganese-oxidizing half-reaction and proton pumping linked to the passage of electrons from manganese to oxygen via the electron transport system in the membrane result in a proton gradient across the membrane (outside acidic relative to the cytoplasm). This gradient contributes to the proton motive force

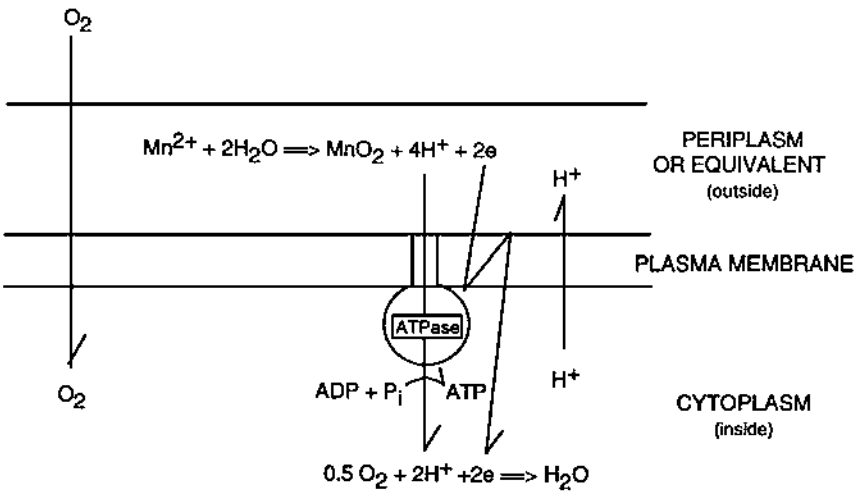


FIG. 16.3 Proposed bioenergetics of Mn^{2+} oxidation by Group Ia bacteria. Note that manganese oxidation occurs in the periplasm in this scheme, whereas oxygen reduction takes place at the inner surface of the plasma membrane. Protons pumped into the periplasm from the cytoplasm by the electron transport system in the plasma membrane (details not shown) together with protons from the oxidative half-reaction of Mn^{2+} contribute to the proton gradient across the membrane and therefore to ATP synthesis. Although the manganese-oxidizing half-reaction is shown as a single reaction in this diagram, it probably occurs in two steps ($Mn^{2+} \rightarrow \{Mn^{3+}\} + e$, and $\{Mn^{3+}\} + 2H_2O \rightarrow MnO_2 + 4H^+ + e$), of which only the second step generates useful energy for the cell (see text).

(PMF) needed for ATP synthesis via ATPase. ADP stimulation of Mn^{2+} oxidation by everted membrane vesicles from strain SSW_{22} is explained by a mass action effect involving vectorial and scalar proton consumption in ATP synthesis. The everted membrane vesicles appeared to become freely permeable to Mn^{2+} in their preparation.

It should be stressed that although the model in Figure 16.3 represents the oxidation of Mn(II) to Mn(IV) as a single half-reaction, namely $Mn^{2+} + 2H_2O \rightarrow MnO_2 + 4H^+ + 2e$, it it should really be seen as the sum of two half-reactions thermodynamically:

$$Mn^{2+} \rightarrow \{Mn^{3+}\} + e \qquad (E'_0 = 0.84\,V) \tag{16.3a}$$

$$\{Mn^{3+}\} + 2H_2O \rightarrow MnO_2 + 2H^+ + e \qquad (E'_0 = +0.08\,V) \tag{16.3b}$$

where $\{Mn^{3+}\}$ represents bound Mn(III), probably in an enzyme complex but possibly combined with a ligand. Reaction (16.3b) is the one that drives ATP synthesis because its E'_0 is low enough to permit entry of the electron into the electron transport chain at the level of a cytochrome bc-type complex, as suggested by experiment. Although oxidation of Mn^{2+} to MnO_2 in a single step [reaction (16.2)] by removal of two electrons is thermodynamically possible ($\Delta G^{\circ\prime} = -16.3$ kcal or 68.1 kJ), its E'_0 is too high (+0.46 V) to allow for ATP synthesis coupled to electron transport. When coupled to the reductive half-reaction for O_2,

$$O_2 + 2H^+ + 2e \rightarrow H_2O \tag{16.3c}$$

the value of $\Delta G^{\circ\prime}$ for the oxidation of Mn^{2+} to $\{Mn^{3+}\}$ is about +1.21 kcal (+5.1 kJ), and for the oxidation of $\{Mn^{3+}\}$ to MnO_2, about −33.8 kcal (141 kJ) (for further discussion see Ehrlich, 1999).

On the basis of a report by Kepkay et al. (1984), *Pseudomonas* strain S-36 should be assigned to Subgroup Ia. This organism binds and oxidizes Mn^{2+} and appears to derive energy from the oxidation of manganese. It may use some of this energy to fix CO_2 (Kepkay and Nealson, 1987). However, Nealson et al. (1988) did not rule out the possibility, even though they considered it remote, that the manganese stimulated carbon utilization by a mechanism other than oxidation.

Subgroup Ib. Subgroup Ib includes bacteria in which manganese oxidation is not directly coupled to ATP synthesis (Fig. 16.4). The only documented example is a gram-negative rod (strain FMn 1) isolated from a body of freshwater (Tomhannock Reservoir, Troy, NY) (Zapkin and Ehrlich, 1983) that oxidizes Mn^{2+} in its periplasm (Zindulis and Ehrlich, 1983). The oxidation involves an as yet unidentified factor that behaves like an oxidant and not like an enzyme (Zindulis and Ehrlich, 1983). The oxidized form of this factor is reduced by Mn^{2+}, and the reduced factor, after passing into the cytoplasm, is then

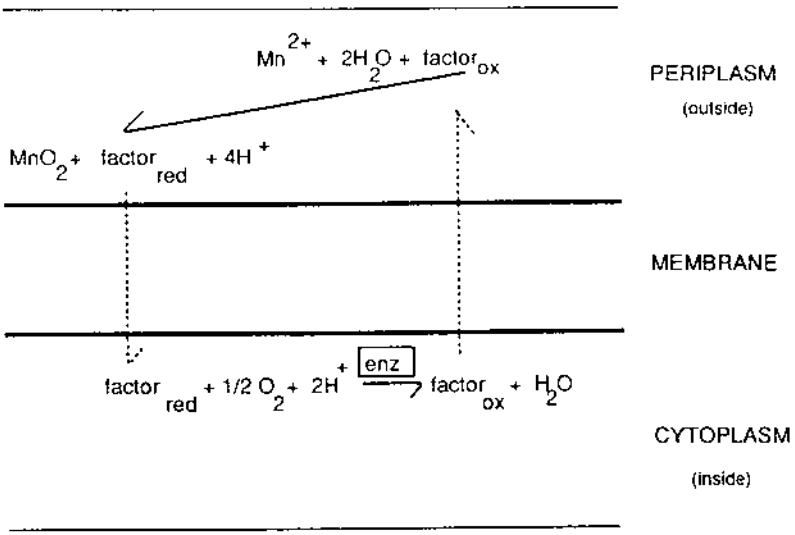


FIG. 16.4 Current model for manganese oxidation by a Group Ib bacterium. The electron transport system of the plasma membrane is not directly involved. Manganese oxidation is the result of interaction with an as-yet-unidentified factor, which is reduced by Mn^{2+}. The reduced factor is reoxidized in the cytoplasm. Whether the protons from the manganese oxidation in the periplasm, if formed as shown, contribute to ATP synthesis is not known.

enzymatically reoxidized with O_2 as terminal electron acceptor. Results from inhibitor studies indicate that the electron transport system in the plasma membrane is not involved (Zindulis and Ehrlich, 1983).

Subgroup Ic. Subgroup Ic includes sheathed bacteria of the genus *Leptothrix*. Early work with *L. discophora* led to contradictory findings. Johnson and Stokes (1966) found that nongrowing (resting) cell suspensions of a strain of *L. discophora* (also called *Sphaerotilus discophorus*) oxidized Mn^{2+} if the culture had been pregrown in a medium supplemented with manganese but not without manganese supplementation. A subsequent study with a different strain of *L. discophora*, however, failed to confirm inducibility of manganese-oxidizing ability in the organism (Van Veen, 1972). A difference in the strains might have been the cause of this difference in results. In another strain of *L. discophora*, manganese-oxidizing activity was found associated with a particulate cell fraction that sediments at $48{,}000 \times g$. It appeared to be coupled to cytochrome oxidase because it was inhibited by 10^{-5} M cyanide and 10^{-4} M azide, but coupling to b- and c-type cytochrome was not observed (Hogan, 1973; Mills

and Randles, 1979). *L. discophora* has been reported to be able to grow autotrophically and mixotrophically on Mn(II) as an energy source (Ali and Stokes, 1971), but these observations were not confirmed (Hajj and Makemson, 1976).

More recent studies with a freshly isolated strain of *L. discophora* (SS-1; ATCC 43182) called the earlier findings into question. Strain SS-1, which has lost the ability to form a sheath but continues to be able to oxidize Mn^{2+} (Adams and Ghiorse, 1986), releases Mn^{2+}-oxidizing protein that has a molecular weight of 100,000–110,000 into liquid culture medium. The protein has a polysaccharide moiety linked to it. The manganese-oxidizing activity of the protein exhibits a pH optimum around 7.5 and a temperature optimum of 25°C. The activity is inhibited by cyanide, *o*-phenanthroline, and $HgCl_2$, but not by azide. The oxidized manganese becomes associated with the protein and can be removed by reduction with ascorbate, thereby restoring the manganese-oxidizing activity of the protein (Adams and Ghiorse, 1987; Boogerd and de Vrind, 1987). Corstjens et al. (1997) identified the *mofA* gene in *L. discophora* SS-1, which codes for a manganese-oxidizing protein that includes copper in its structure and is related to multicopper oxidases. Addition of Cu^{2+} to the culture medium was found to stimulate production of the manganese-oxidizing protein in growing cultures of *L. discophora* SS-1 although it decreased the cell yield (Brouwers et al., 2000b). The excretion of a manganese-oxidizing protein by *Leptothrix* spp. was first reported by Mulder (1972) and Van Veen (1972).

Adams and Ghiorse reported in 1988 that the excreted protein acts as a catalyst in association with an acidic exopolymer in oxidizing Mn(II) to Mn(III). The formation of Mn in the +3 oxidation state yields only $\sim 10\,\text{kcal mol}^{-1}$ at pH 7.0 (assuming hausmannite as the product) under otherwise standard conditions, compared to the oxidation of Mn(II) to Mn(IV), which yields $\sim 16\ \text{kcal mol}^{-1}$ at pH 7 under otherwise standard conditions. Excess concentrations of Mn^{2+} inhibit the growth of *L. discophora* SS-1, probably because the manganese oxide (MnO_2) that it deposits on the cells in the absence of a sheath deprives it of essential iron by binding it (Adams and Ghiorse, 1985).

Subgroup Id. Subgroup Id currently accommodates marine *Bacillus* SG-1 (Nealson and Ford, 1980). The vegetative cells of this organism do not oxidize Mn(II), but its dormant spores do (Rosson and Nealson, 1982; de Vrind et al., 1986b; Francis and Tebo, 1999). The formation of the spores by the vegetative cells of the bacillus is enhanced by solid surfaces in dilute seawater medium (Kepkay and Nealson, 1982). The spores bind and oxidize Mn^{2+} (Rosson and Nealson, 1982). The bound Mn^{2+} is oxidized by a protein component in the exosporium (de Vrind et al., 1986b; Francis and Tebo, 1999). The protein is actually a complex of several smaller proteins. The complex has a molecular mass of 205 kDa and may be a glycoprotein (Tebo et al., 1988), but according to

Francis and Tebo (1999) these results have been difficult to reproduce. An operon, labeled *mnx*, consisting of genes *mnxA* to *mnxG* on the chromosome of *Bacillus* SG-1, codes for factors required for manganese oxidation by the spores (Van Waasbergen et al., 1993, 1996). The gene *mnxG* codes for a protein that belongs to the multicopper oxidase family. The product of gene *mnxC* may be a redox active protein involved in the oxidation of Mn^{2+}, but it may also be involved in Cu^{2+} transport into the cell (Tebo et al., 1997). Addition of Cu^{2+} to the growth medium stimulates manganese oxidation by the spores (Van Waasbergen et al., 1996), consistent with the notion that the oxidation involves a multicopper oxidase. The initial product of manganese oxidation by the spores was first thought to be Mn_3O_4 (hausmannite) (Hastings and Emerson, 1986) but was later identified as Mn(IV) oxide (10 Å manganate) (Mandernak et al., 1995). The direct formation of Mn(IV) oxide from Mn(II) was confirmed in an application of X-ray absorption near-edge structure spectroscopic analysis of the spores when oxidizing Mn^{2+}. No evidence of Mn(III) formation, even as an intermediate, was detected (Bargar et al., 2000). Francis and Tebo (1999) suggest that the Mn_3O_4 detected on spores in early work may have been the product of Mn(II) oxidation catalyzed abiotically by enzymatically formed Mn(IV) oxide on the spore surface and thus would be the product of secondary reaction, as previously suggested by de Vrind et al. (1986b) and Mann et al. (1988). Because the products of manganese oxidation by the spores remain bound to the spore surface, it remains unclear whether the spores can sustain continuous enzymatic manganese oxidation.

Spores produced by a strain of *Bacillus megaterium* (strain BC1), which was isolated from a microbial mat at Laguna Figueroa, Baja California, can also oxidize Mn^{2+}. They are thus similar to those formed by *Bacillus* SG-1 (Gong-Collins, 1986).

Whereas vegetative cells of *Bacillus* SG-1 cannot oxidize Mn^{2+}, de Vrind et al. (1986a) discovered that they can reduce MnO_2 at low oxygen tension. The electron donor in these organisms is an unidentified intracellular compound; externally supplied glucose or succinate did not act as electron donor. MnO_2 reduction involved a branched, membrane-bound electron transport pathway in which oxygen at normal tension competed with MnO_2 as terminal electron acceptor. The MnO_2-reducing activity in this organism is thought to supply needed Mn^{2+} for sporulation in environments where the supply of Mn^{2+} is limited or absent but where manganese oxide is available (de Vrind et al., 1986a).

The oxidation of Mn(II) by spores but not by vegetative cells of members of the genus *Bacillus* is by no means a universal phenomenon with spore formers. Ehrlich and Zapkin (1985) and Vojak et al. (1984) isolated Bacilli whose oxidation of Mn(II) depended on vegetative cells, in the latter instance cells that were in the process of sporulating. These organisms should be assigned to Subgroup Ia based on knowledge to date.

Uncertain Subgroup Affiliation. Subgroup assignment of *Pseudomonas putida* strains MnB1 and GB-1, both gram-negative, is problematic. These two cultures do show some affinity to Subgroup Ia based on characterizations to date (DePalma, 1993; Okazaki et al., 1997; Fig. 7 in Tebo et al., 1997; Brouwers et al., 2000a). However, it is presently unclear whether these strains can conserve energy from manganese oxidation. Their manganese oxidase appears to be a protein complex that includes a multicopper oxidase (Brouwers et al., 1999) and appears to reside in the outer membrane, because Mn^{2+} oxidation occurs at the cell surface (Okazaki et al., 1997; Brouwers et al., 2000a).

Subgroup assignment within Group I of manganese-oxidizing *Pseudomonas* strain E1 and *Citrobacter freundii* strain E4, isolated from manganese concretions found in an alfisol soil in West Peleponnesus, Greece (Douka, 1977, 1980; Douka and Vaziourakis, 1982), is also problematic. It was previously assigned to Subgroup Id (Ehrlich, 1996a), but based on the current definition of the other subgroups, the information on these organisms is insufficient for subgroup assignment. Oxidation by the two *Pseudomonas* and *Citrobacter* strains was demonstrated with intact cells and cell extracts. The activity was heat-sensitive and inhibited by $HgCl_2$. Cell extracts exhibited a temperature optimum at 34°C. Whether these bacteria can derive energy from manganese oxidation remains to be determined.

Group II Manganese Oxidizers

Group II of manganese oxidizers includes those bacteria that oxidize Mn^{2+} only when it is prebound to one of several solids external to the cell. Like Group I bacteria, they use oxygen as terminal electron acceptor. At least some bacteria using this mechanism can gain useful energy from the oxidation. The oxidative reaction catalyzed by these bacteria when Mn^{2+} is bound to a hydrated Mn(IV) oxide like $MnO_2 \cdot H_2O$ (H_2MnO_3) can be summarized as follows:

$$MnMnO_3 + 0.5O_2 + 2H_2O \rightarrow 2H_2MnO_3 \quad (16.4a)$$

The $MnMnO_3$ results from a reaction in which Mn^{2+} displaces the protons of H_2MnO_3:

$$Mn^{2+} + H_2MnO_3 \rightarrow MnMnO_3 + 2H^+ \quad (16.4b)$$

Reaction (16.4a) is catalyzed by the bacteria on the surface of the $MnMnO_3$ particles and is the rate limiting step in the overall oxidation of Mn(II) to Mn(IV) in this process. Reaction (16.4b) is very rapid and not catalyzed by the bacteria. It is oxygen-independent.

Those Group II organisms whose ability to oxidize manganese(II) has been examined in some detail perform the oxidation in their periplasm (gram-negative bacteria) or their periplasmic equivalent (gram-positive bacteria) like the bacteria in Group I, Subgroup 1a. They differ from Subgroup 1a organisms by attacking

only Mn^{2+} that is prebound to a solid substrate such as Mn(IV) oxide, ferromanganese, or clays such as montmorillonite or kaolinite, but not illite (Arcuri, 1978; Arcuri and Ehrlich, 1979; Ehrlich, 1982, 1984). In the case of oxidation of Mn^{2+} bound to clays, pretreatment of the clays overnight with ferric chloride in the absence of added Mn^{2+} was essential for whole cells to oxidize manganese(II) that was subsequently bound to clays (Ehrlich, 1982). Such ferric chloride pretreatment was not necessary when cell extracts were used, but the amount of manganese oxidized in 4 hr was less without than with ferric chloride pretreatment. The iron was not a factor required for bacterial activity, because when ferric chloride and manganese were added simultaneously to the reaction mixture the bacteria did not oxidize the bound manganese. The iron seems to play a steric role in making bound Mn^{2+} more accessible to oxidation by the cells or cell extract. So far it is unclear how the Mn^{2+} bound to an appropriate solid inorganic phase outside the cell is transported into its periplasm or its equivalent. At least one periplasmic factor has been detected that is essential to manganese oxidation by Group II organisms (Arcuri and Ehrlich, 1979, 1980). However, it remains to be determined whether it is involved in transferring Mn^{2+} into the periplasm or whether it is involved in electron transfer to the electron transport system in the plasma membrane. Like members of Subgroup Ia, Group II manganese-oxidizing bacteria can couple ATP synthesis to Mn(II) oxidation (Ehrlich, 1976) by using a respiratory chain in the plasma membrane (Arcuri and Ehrlich, 1979). A diagram of a working model for manganese oxidation by Group II bacteria is shown in Figure 16.5. As in Subgroup Ia bacteria, the oxidation of Mn^{2+} to MnO_2 is assumed to proceed in two steps, of which only the second step yields enough energy to be conserved.

Group III Manganese Oxidizers

Group III manganese oxidizers include those bacteria that oxidize dissolved Mn(II) with H_2O_2 using catalase as the enzyme that promotes this reaction. The reaction can be summarized as follows:

$$Mn^{2+} + H_2O_2 \rightarrow MnO_2 + 2H^+ \tag{16.5}$$

Group III bacteria so far include *Metallogenium*, *Leptothrix pseudoochracea*, and *Siderobacter capsulatus* (Dubinina, 1978a). These organisms appear to generate H_2O_2 faster in their metabolism than their catalase can destroy it by the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2 \tag{16.6}$$

In the presence of Mn^{2+} at neutral pH, their catalase can function as a peroxidase and use H_2O_2 as an oxidant to generate MnO_2 according to reaction (16.5) (Dubinina, 1978b). The reaction can be reproduced in the laboratory by generating H_2O_2 from glucose oxidation by glucose oxidase and then causing the H_2O_2

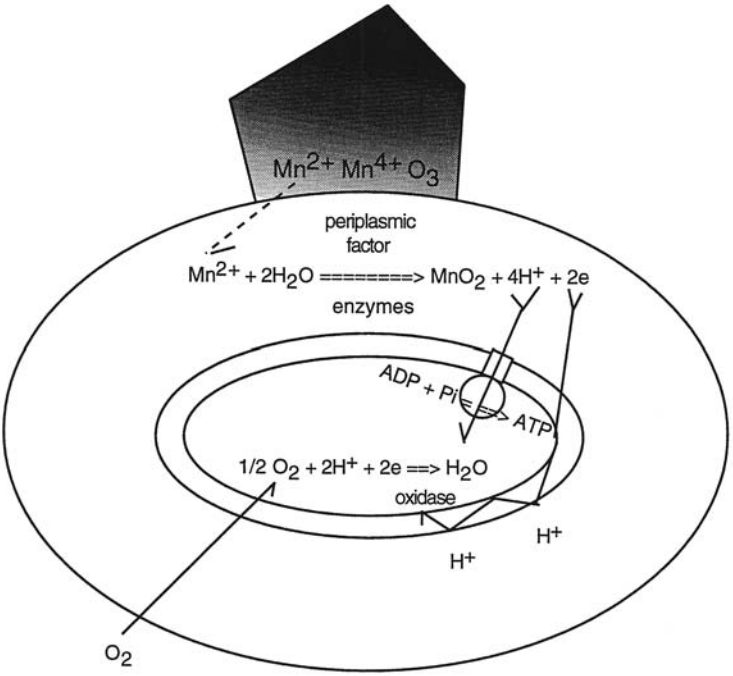


FIG. 16.5 Proposed bioenergetics of $MnMnO_3$ oxidation by Group II bacteria. Note that this scheme is similar to that for Group Ia bacteria, except that the Mn^{2+} derives from bound Mn(II). As in the case of Group Ia bacteria, the manganese oxidizing half-reaction represents the sum of two steps in the oxidation of Mn^{2+} to MnO_2 (see legend of Fig. 16.3).

to oxidize Mn^{2+} with a commercial preparation of catalase (Dubinina, 1978a). The oxidation product was identified as an Mn(IV) compound, but because it was complexed by pyrophosphate it could have been an Mn(III) compound. Fe^{2+} can replace Mn^{2+} in this reaction (Dubinina, 1978b). At acid pH, H_2O_2 can reduce MnO_2 to Mn^{2+} chemically according to the reaction

$$MnO_2 + H_2O_2 + 2H^+ \rightarrow Mn^{2+} + 2H_2O + O_2 \qquad (16.7)$$

This reaction proceeds without catalase.

Nonenzymatic Manganese Oxidation

Autoxidation of Mn^{2+} is promoted when environmental conditions feature an E_h greater than +500 mV, a pH greater than 8, and an Mn^{2+} concentration greater than 0.01 ppm. These pH and E_h limits are much narrower than those required for

autoxidation of iron (see Chap. 15). Nonenzymatic manganese oxidation may also be promoted through production of one or more metabolic end products, which cause chemical oxidation of Mn^{2+}. According to Soehngen (1914), a large number of microorganisms can cause such manganese oxidation when in the presence of hydroxycarboxylic acids such as citrate, lactate, malate, gluconate, or tartrate. He indicated that metabolic utilization of a part of such acids, or more exactly their salts, causes a rise in pH of the culture medium (if unbuffered), and that when the pH turns alkaline, residual hydroxycarboxylic acid catalyzes the oxidation of Mn^{2+}. Hydroxycarboxylic acids can be formed by both bacteria and fungi. In apparent agreement, Van Veen (1973) found that with *Arthrobacter* 216, hydroxycarboxylic acids are required for Mn^{2+} oxidation. However, he felt that other microorganisms with more specific manganese-oxidizing capacity play a more important role in manganese oxidation in soil.

Another example of nonenzymatic manganese oxidation is furnished by an actinomycete, *Streptomyces* sp., found in Australian soil. In the laboratory it causes Mn^{2+} to be oxidized in a pH range of 5–6.5 when growing in soil agar (Bromfield, 1978, 1979). The actinomycete produces a water-soluble extracellular compound that is responsible for the oxidation. The oxidized manganese is thought to protect the organism from inhibition by Mn^{2+} ions (Bromfield, 1978).

Pseudomonas manganoxydans has been reported to form an Mn^{2+}-oxidizing protein that oxidizes Mn(II) intracellularly and is consumed in the process (Jung and Schweisfurth, 1979). On the basis of this observation, the authors concluded that the protein is not an enzyme. They also found that this oxidation did not require oxygen. It proceeded optimally at pH 7.0. The formation of the protein depended on cessation of growth by the culture at the end of its exponential phase but did not require added Mn^{2+} in the medium to stimulate its synthesis (Jung and Schweisfurth, 1979). Later study of this culture by DePalma (1993) and Okazaki et al. (1997) led to its reclassification as *Pseudomonas putida* strain MnB1. These investigators also demonstrated that the strain did possess a manganese-oxidizing enzyme system (see above: Group I, uncertain subgroup affiliation).

Zygospores of the alga *Chlamydomonas* from soil have been reported to become encrusted with Mn(IV) oxide, presumably through oxidation of Mn^{2+} (Schulz-Baldes and Lewin, 1975), but how the manganese came to be oxidized was not explained. It could have been a case of passive accumulation of manganese oxide (see Sec. 16.7).

16.6 BIOREDUCTION OF MANGANESE

Microbial reduction of oxidized manganese can be enzymatic or nonenzymatic, as in the case of microbial oxidation of manganese. The occurrence of microbial

reduction of manganese oxide was suggested as far back as the 1890s by Adeny, (1894), who found that manganese dioxide, formed when he added permanganate to sewage, was reduced to manganous carbonate. He attributed this reduction to the action of bacteria and thought it analogous to bacterial nitrate reduction. Mann and Quastel (1946) reported the microbial reduction of biogenically formed manganese oxide in a soil perfusion column when it was fed with glucose solution. They were able to inhibit this reaction by feeding the column with azide. They suggested that the oxides of manganese acted as hydrogen acceptors. Troshanov (1968) isolated a number of Mn(IV)-reducing bacteria from reduced horizons of several Karelian lakes (former U.S.S.R.). His isolates included *Bacillus circulans*, *B. polymyxa*, *B. mesentericus*, *B. mycoides*, *B. cereus*, *B. centrosporus*, *B. filaris*, *Pseudomonas liquefaciens*, and *Micrococcus* sp., among others. Some of these strains were able to reduce both manganese and iron oxides, but strains that reduced only manganese oxides were encountered most frequently. Nitrate did not inhibit Mn(IV) reduction the way it did Fe(III) reduction by *B. circulans*, *B. polymyxa*, *B. mesentericus*, and *Ps. liquefaciens* (Troshanov, 1969). Anaerobic conditions stimulated Mn(IV) reduction by *B. centrosporus*, *B. mycoides*, *B. filaris*, and *B. polymyxa* but not by *B. circulans*, *B. mesentericus*, or *Micrococcus albus*. Not all carbohydrates tested were equally good sources of reducing power for a given organism (Troshanov, 1969). Although a significant part of the Mn(IV) seemed to be reduced enzymatically, some of it appeared to be reduced chemically, because some reduction of oxidized manganese in lake ores by glucose and xylose and some organic acids (e.g., acetic and butyric acids) was observed at pH 4.3–4.4 under sterile conditions (Troshanov, 1968). Such reaction of glucose and other organic reducing agents with Mn(IV) oxides is, however, dependent on the form of the oxide; hydrous Mn(IV) oxides react readily, whereas crystalline MnO_2 does not (see, e.g., Ehrlich, 1984; Nealson and Saffarini, 1994).

Bromfield and David (1976) reported that *Arthrobacter* strain B, which can oxidize Mn(II) aerobically, is able to reduce manganese oxides at lowered oxygen tension. Ehrlich (1988) reported that several gram-negative bacteria that can oxidize Mn(II) and thiosulfate aerobically and reduce tetrathionate anaerobically (Tuttle and Ehrlich, 1986) can also reduce MnO_2 aerobically and anaerobically.

Clearly, a variety of microbes have the capacity to reduce oxidized manganese. Some bacteria can reduce Mn(IV) oxide aerobically or anaerobically (e.g., Ehrlich, 1966; Di-Ruggiero and Gounot, 1990; Troshanov, 1968). Others reduce it only anaerobically (e.g., Burdige and Nealson, 1985; Lovley and Phillips, 1988a; Myers and Nealson, 1988a). Among these latter bacteria, some, like *Shewanella* (*Alteromonas, Pseudomonas*) *putrefaciens*, are facultative but nevertheless reduce Mn(IV) oxide only anaerobically (Myers and Nealson, 1988a), whereas others, like *Geobacter metallireducens* (formerly strain GS-15), are strict anaerobes and reduce MnO_2 only anaerobically (Lovley and Phillips, 1988a).

Reduction of Manganese Oxides by Organisms Capable of Performing It Aerobically and Anaerobically

A number of different bacterial cultures have been isolated from marine and freshwater environments that can reduce Mn(IV) oxide aerobically and anaerobically. As in the case of enzymatic oxidation of Mn(II) by bacteria, the marine cultures do not all employ the same electron transport pathway in enzymatic reduction of oxidized manganese (Ehrlich, 1980, 1993b). The freshwater isolates have not yet been tested in this regard. Growing cultures of marine *Bacillus* 29 are able to reduce MnO_2 aerobically and anaerobically using glucose as electron donor. However, a component of the electron transport system involved in MnO_2 reduction in this culture is only aerobically inducible (Trimble and Ehrlich, 1968). Both Mn^{2+} and MnO_2 can serve as inducers. Because of the aerobic requirement for induction, uninduced *Bacillus* 29 cannot reduce MnO_2 anaerobically unless an artificial electron carrier such as ferricyanide is added (Ehrlich, 1966; Trimble and Ehrlich, 1968, 1970). Once initiated by a growing culture of *Bacillus* 29, MnO_2 reduction is quickly inhibited by addition of $HgCl_2$ to a final concentration of 10^{-3} M. During MnO_2 reduction with glucose as electron donor, the concentrations of pyruvate and lactate formed by the organism from glucose were greater in the presence than in the absence of MnO_2 by day 11 (Table 16.4). However, calculations from the data in Table 16.4 show that on the basis of 100 nmol of glucose consumed, *Bacillus* 29 formed 7.9 nmol pyruvate with MnO_2 but only 3.1 nmol without it by day 11. Yet it formed 20.4 nmol lactate with MnO_2 and 24.2 nmol without it per 100 nmol of glucose consumed in the same length of time. The organism consumed more glucose in the presence of MnO_2 than in its absence (Table 16.4). Glucose consumption and total product formation qualitatively similar to those with growing cultures of *Bacillus* 29 occurred with marine coccus 32 (Trimble and Ehrlich, 1968).

Aerobic MnO_2 reduction has also been studied with resting cell cultures and with cell extracts. In the case of *Bacillus* 29, uninduced resting cells were able to reduce MnO_2 only in the presence of dilute ferri- or ferrocyanide, whereas induced resting cells were able to reduce MnO_2 without ferri- or ferrocyanide. The ferri- or ferrocyanide substituted for a missing component in the electron transport system to MnO_2 in uninduced cells (Ehrlich, 1966; Trimble and Ehrlich, 1970; Ghiorse and Ehrlich, 1976). Substrate level concentrations of ferrocyanide can serve as electron donor in place of glucose in the reduction of MnO_2 by *Bacillus* 29 (Ghiorse and Ehrlich, 1976). Mann and Quastel (1946) had previously observed that pyocyanin could serve as electron carrier in MnO_2 reduction by bacteria, but they believed that such a carrier was essential for such reduction to occur.

TABLE 16.4 Chemical and Physical Changes During Growth[a] of *Bacillus* 29 and coccus 32 in the Presence and Absence of MnO_2

Culture	Time (days)	Glucose consumed (nmol mL^{-1})		Manganese released (nmol mL^{-1})	Pyruvate produced (nmol mL^{-1})		Lactate produced (nmol mL^{-1})		E_h (mV)		pH		Cells mL^{-1} ($\times 10^7$)	
		$+MnO_2$	$-MnO_2$	$+MnO_2$	$+MnO_2$	$-MnO_2$	$+MnO_2$	$-MnO_2$	$+MnO_2$	$-MnO_2$	$+MnO_2$	$-MnO_2$	$+MnO_2$	$-MnO_2$
29	0	0	0	0	0	0	0	0	469	474	7.3	7.2	1.6	1.6
	1	1720	655	25	43	32	461	449	528	482	6.6	6.7	3.8	4.0
	3	3060	1239	240	190	153	1635	1612	502	512	5.4	5.6	4.5	4.8
	4	3686	2342	420	438	330	1430	1955	573	514	5.7	5.5	4.5	5.0
	7	6120	3635	1060	705	243	2080	1387	615	594	5.7	5.5	4.6	5.2
	8	8750	5286	1260	879	330	2040	1705	617	589	5.5	4.9	4.8	5.0
	11	11893	7281	1830	936	225	2430	1761	602	589	5.8	5.1	5.0	5.0
32	0	0	0	0	0	0	0	0	426	426	7.7	7.7	2.5	2.5
	1	480	0	0	50	52	0	0	394	419	7.0	6.9	4.6	5.8
	2	1440	1440	0	84	75	9	5	519	490	6.8	6.5	6.2	7.0
	3			130	138	79			491	496	6.6	6.4	6.2	7.0
	7	3000	2650	490	1025	575	174	68	484	495	5.7	4.7	6.0	4.8
	9	4900	3125	780	1650	980	250	124	514	549	5.4	4.5	5.8	4.2
	11	5550	3600	1020	2625	1120	250	123	475	509	5.6	4.3	5.4	4.8

[a] Culture medium: For *Bacillus* 29, 0.48% glucose and 0.048% peptone in seawater; for coccus 32, 0.60% glucose and 0.048% peptone in seawater. One gram portions of reagent grade MnO_2 were added to 20 mL of medium as needed. Incubation was at 25°C.
Source: Trimble and Ehrlich (1968), with permission.

Induction of the missing electron transport component involves protein synthesis, because inhibitors of protein synthesis prevent its induction by manganese (Trimble and Ehrlich, 1970). Reduction of MnO_2 by cell extracts from *Bacillus* 29 proceeds in the same way as with resting cells (Ghiorse and Ehrlich, 1976). Extracts from cells previously grown without added $MnSO_4$ do not reduce MnO_2 in the absence of added small amounts of ferri- or ferrocyanide, whereas extracts from cells previously grown in the presence of added $MnSO_4$ do. Results from spectrophotometric and inhibitor studies of the electron transport components in the plasma membrane of *Bacillus* 29 led initially to the inference that electron transfer from electron donors to MnO_2 in induced cells involved a branched pathway. It consisted of flavoproteins, c-type cytochrome, and two metalloproteins, one an MnO_2 reductase and the other a conventional oxidase (Fig. 16.6a). The existence of two metalloproteins was initially postulated

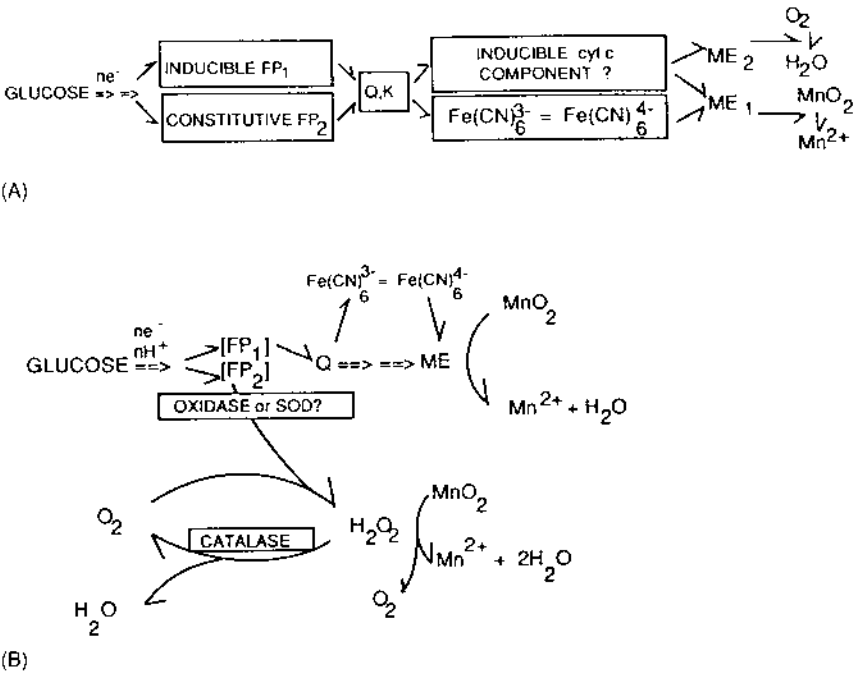


FIG. 16.6 Electron transfer pathways in MnO_2 reduction by *Bacillus* 29. (A) Original proposal. (Redrawn with permission from Ghiorse and Ehrlich, 1976.) FP_1, flavoprotein 1; FP_2, flavoprotein 2; Q,K, coenzyme Q; ME_1, metalloprotein 1 (MnO_2 reductase); ME_2, metalloprotein 2 (an oxidase such as cytochrome oxidase). (B) Amended proposal. (Modified from Ghiorse, 1988.) FP_1, flavoprotein; FP_2, glucose oxidase; SOD, superoxide dismutase (converts superoxide radicals formed during oxygen reduction to H_2O_2 and O_2); Q, coenzyme Q; ME, metalloprotein (MnO_2 reductase).

because MnO_2-reducing activity was stimulated by sodium azide at 1.0–10 mM concentrations. The oxidase was presumed to be more azide sensitive than the MnO_2 reductase (Ghiorse and Ehrlich, 1976). Subsequent observations, however, led to the conclusion that the azide stimulation in *Bacillus* 29 was the result of inhibition of catalase activity with a resultant reduction of some MnO_2 by accumulating H_2O_2 [see reaction (16.7)] (Fig. 16.6b) (Ghiorse, 1988). This reduction by metabolically produced H_2O_2 is similar to that previously reported by Dubinina (1979a, 1979b) with *Leptothrix pseudoochracea*, *Arthrobacter siderocapsulatus*, and *Metallogenium*.

The organization of the MnO_2-reducing system in *Bacillus* 29 is not universal in manganese reducing bacteria. In other marine MnO_2-reducing bacteria so far examined, electron transport appears to involve more conventional electron transport carriers and enzymes, as suggested by electron transport inhibitor studies. These inhibitor studies, moreover, indicated that the pathway appears not to be exactly the same in each of the organisms tested (Ehrlich, 1980, 1993b). H_2O_2 appears to play no significant role in MnO_2 reduction by these organisms because 1 mM azide did not stimulate MnO_2 reduction. Ghiorse (1988) suggested that in *Bacillus* 29, MnO_2 reduction may serve as a means of disposing of excess reducing power without energy coupling. In strains involving more conventional electron transport components for the reduction of MnO_2, such reduction in air may be a supplemental source of energy.

How is it that MnO_2 can partially replace oxygen as terminal electron acceptor for some aerobic bacteria? *Bacillus* 29 was found to consume less oxygen in the presence of MnO_2 than in its absence when respiring on glucose (Trimble, 1968; Ehrlich, 1981). MnO_2 reduction by *Bacillus* GJ33 was accelerated in rotary-shake culture in air at 200 rpm but depressed at 300 rpm (Ehrlich, 1988). This can be explained thermodynamically (Ehrlich, 1987). Considering that the standard free energy change at pH 7.0 ($\Delta G^{\circ\prime}$) for the half-reaction

$$MnO_2 + 4H^+ + 2e \rightarrow Mn^{2+} + 2H_2O \tag{16.8}$$

is only -18.5 kcal (-77.3 kJ), and that for the half-reaction

$$0.5O_2 + 2H^+ + 2e \rightarrow H_2O \tag{16.9}$$

at pH 7.0 it is -37.6 kcal (-157 kJ), it might be concluded that oxygen should be the better electron acceptor and therefore MnO_2 should not depress its consumption. However, the standard free energy values above are for standard conditions at pH 7.0 (1 M solute concentrations, except for H^+, and 1 atm for gases). These conditions do not apply to laboratory experiments or in nature. Oxygen concentration in pure water at atmospheric pressure and 25°C is less than $10^{-2.89}$ M, whereas the concentration of Mn(IV) in MnO_2 that the bacteria encounter suspended in water is orders of magnitude greater than 1 M because MnO_2 is insoluble in water. Because of its insolubility, the bacteria must be in

physical contact with the MnO_2 (see discussion by Ghiorse, 1988). The specific mechanism by which bacteria are able to transfer electrons to insoluble MnO_2 remains to be worked out, but possible models for aerobic and anaerobic reduction are presented below. The problem must be similar to that encountered when bacteria oxidize very insoluble metal sulfides by direct action (see Chap. 19).

The ability to reduce MnO_2 was found to be inducible in all marine isolates tested by Ehrlich (1973). Despite differences in the electron transport pathway from donor to recipient, the overall reaction involving MnO_2 reduction appears to be the same in all marine cultures studied by Ehrlich and collaborators (Ehrlich et al., 1972; Ehrlich, 1973). Considering glucose as electron donor, it may be summarized as follows:

$$\text{Glucose} \xrightarrow{\text{bacteria}} ne + nH^+ + \text{end products} \tag{16.10}$$

$$\frac{n}{2}MnO_2 + ne + nH^+ \xrightarrow[\substack{\text{or uninduced bacteria}\\ +\text{ferri- or ferrocyanide}}]{\text{induced bacteria}} \frac{n}{2}Mn(OH)_2 \tag{16.11}$$

$$\frac{n}{2}Mn(OH)_2 + nH^+ \rightarrow \frac{n}{2}Mn^{2+} + nH_2O \tag{16.12}$$

The reason for representing the direct product of MnO_2 reduction by the bacteria as $Mn(OH)_2$ rather than Mn^{2+} is that in resting cell experiments or in experiments with cell extract that were run for only 3–4 hr, it was necessary to acidify the reaction mixture to about pH 2 on completion of incubation in order to bring Mn^{2+} into solution. No manganese was solubilized by acidification at time 0 from the MnO_2 employed in these experiments. The acidification was not necessary in growth experiments, in which acid production from glucose by the bacteria or complexation by medium constituents bring Mn^{2+} into solution (Trimble and Ehrlich, 1968).

The enzyme directly responsible for MnO_2 reduction by organisms that do it aerobically and anaerobically has so far not been isolated and characterized. In the case of *Acinetobacter calcoaceticus*, assimilatory nitrate reductase has been implicated (Karavaiko et al., 1986). MnO_2 reduction by cell extract from this organism was found to be inhibited by NH_4^+ and NO_3^-. The reduction was stimulated if the cells had been cultured in the presence of added molybdenum or if molybdate was added to the reaction mixture in which MnO_2 was being reduced with extract from cells grown in medium without added molybdenum (Karavaiko et al., 1986). MnO_2-reducing activity by *A. calcoaceticus* appears to be assisted by the simultaneous production of organic acids by the organism (Yurchenko et al., 1987).

Ehrlich (1993a, 1993b) has proposed a model (Fig. 16.7) to explain how bacteria are able to reduce insoluble MnO_2 either aerobically or anaerobically

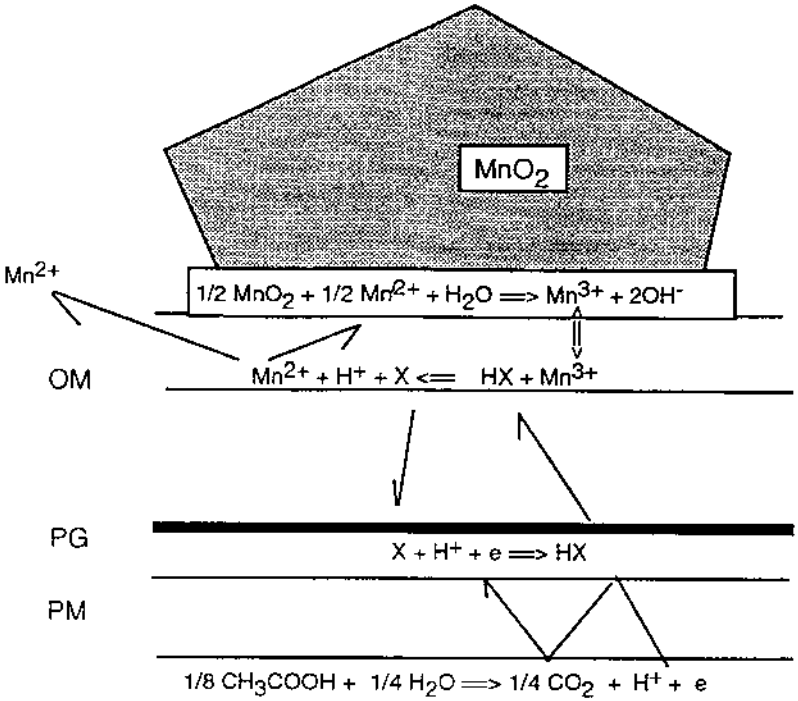


FIG. 16.7 Schematic representation of a model explaining the transfer of reducing power (electrons) across the interface between the cell surface of marine pseudomonad BIII88 and the surface of an MnO_2 particle with which the bacterium is in physical contact. OM, outer cell membrane; PG, peptidoglycan layer; PM, plasma membrane; X, hypothetical carrier of reducing power in the cell envelope. (From Ehrlich, 1993b, with permission.)

using acetate as electron donor. This model requires that the bacteria be in direct physical contact with MnO_2 particles. Mn^{2+} bound in the outer cell envelope enters into a disproportionation reaction with MnO_2 at the site of attachment to the particle [reaction (16.1)]. The oxidation state of the bound Mn^{2+} is thereby raised to Mn^{3+} and that of the reacting Mn(IV) in the MnO_2 particle is lowered to Mn^{3+}. Reducing power removed by the bacteria from electron donors (acetate in the example) is transferred by enzymes and carriers to the Mn^{3+} in their outer cell envelope, re-reducing it to Mn^{2+}. Most of this reduced Mn^{2+} is released into the bulk phase, but some remains bound in the outer cell envelope to continue the disproportionation of the MnO_2 of the particle. The model is based on the following experimental observations with marine pseudomonad BIII 88 (Ehrlich, 1993a).

The plasma membrane of marine pseudomonad BIII 88 contains a respiratory system that includes b and c cytochromes. Aerobically, MnO_2 reduction with acetate by intact cells was stimulated by electron transport inhibitors antimycin A and 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), suggesting a branched respiratory pathway to the terminal electron acceptors MnO_2 and O_2, the branch to O_2 being more sensitive to the inhibitors than the branch to MnO_2. Anaerobically, both inhibitors caused a decrease in MnO_2 reduction. Oxidative phosphorylation uncouplers 2,4-dinitrophenol and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) stimulated MnO_2 reduction at critical concentrations aerobically and anaerobically, indicating that energy is conserved in MnO_2 reduction. Induced cells contain significantly more manganese in their cell envelope than uninduced cells. The extent of their MnO_2-reducing activity was strongly correlated with the manganese concentration in their cell envelope. Cell envelopes of marine bacterial strains that could oxidize Mn^{2+} but not reduce MnO_2 or that could neither oxidize Mn^{2+} nor reduce MnO_2 contained less manganese and exhibited marginal or no MnO_2-reducing activity.

This model helps to explain why marine pseudomonad BIII 88, unlike *Geobacter metallireducens* or *Shewanella putrefaciens*, which reduce MnO_2 only anaerobically, can reduce MnO_2 aerobically as well as anaerobically. It depends on the stability of dissolved Mn^{2+} in air at circumneutral and weakly acid pH in the absence of a catalyst, as opposed to Fe^{2+}, which would rapidly autoxidize. Bacteria that can reduce MnO_2 only anaerobically appear to use an oxygen-sensitive Fe(II)/Fe(III)-containing transfer agent (most likely cytochrome, according to recent evidence; see next section) between MnO_2 and the cell envelope.

Enzymatic Reduction of Manganese Oxides Restricted to Anaerobic Conditions

Bacterial reduction of manganese oxides such as MnO_2 that occurs only anaerobically has been well documented (Burdige and Nealson, 1985; Lovley and Phillips, 1988a; Lovley and Goodwin, 1988; Myers and Nealson, 1988a; Lovley et al., 1989; 1993b). Organisms that are facultative aerobes but that reduce Mn(IV) oxide only anaerobically in an anaerobic respiratory process include *Shewanella* (formerly *Alteromonas*, *Pseudomonas*) *putrefaciens* (Myers and Nealson, 1988a), *Pantoea agglomerans* SP1 (Francis et al., 2000), *Bacillus polymyxa* D1, and *Bacillus* MBX1 (Rusin et al., 1991a, 1991b). Organisms that are strict anaerobes and reduce Mn(IV) oxide in an anaerobic respiratory process include *Geobacter metallireducens* (formerly strain GS-15) (Lovley and Phillips, 1988a), *G. sulfurreducens* (Caccavo et al., 1994), *Geothrix fermentans* (Coates et al., 1999), *Pyrobaculum islandicum* (Kashefi and Lovley, 2000), *Sulfurospirillum barnesii* SES-3 (Laverman et al., 1995), *Desulfovibrio desulfuricans*, *Desulfomicrobium baculatum*, *Desulfobacterium autotrophicum*, and

Desulfuromonas acetoxidans (Lovley and Phillips, 1994a). All but one of these organisms, *P. islandicum*, belong to the domain Bacteria. All are quite versatile in regard to the electron donors they can utilize for MnO_2 reduction. For instance, *G. metallireducens* can use butyrate, propionate, lactate, succinate, and acetate and several compounds, which are all completely oxidized to CO_2 (Lovley and Phillips, 1988a; Lovley, 1991; Myers et al., 1994; Langenhoff et al., 1997). *S. putrefaciens*, on the other hand, can utilize lactate and pyruvate as electron donors but oxidizes these only to acetate. It can, however, use H_2 and formate as electron donors whereas *G. metallireducens* cannot (Lovley et al., 1989). Unlike *G. metallireducens*, *G. sulfurreducens* can use H_2 as an electron donor (Caccavo et al., 1994). As described in Chapter 15, these organisms are also not restricted to MnO_2 as terminal electron acceptor anaerobically. Depending on which organism is considered, Fe(III), uranyl ion, chromate, nitrate, iodate, elemental sulfur, sulfite, sulfate, thiosulfate, fumarate, and glycine may serve. In the case of *S. putrefaciens*, oxygen, nitrate, and fumarate inhibited MnO_2 reduction but sulfate, sulfite, molybdate, nitrite, or tungstate did not (Myers and Nealson, 1988a).

Anaerobic utilization of acetate as electron donor in reduction of MnO_2 is proof of anaerobic respiration. This is because acetate is unfermentable except in the special case of methanogenesis. Because acetate can also be respired in air where oxygen can serve as terminal electron acceptor, it is interesting to note that gram-negative marine strain SSW_{22} can reduce MnO_2 with acetate, succinate, and glucose aerobically as well as anaerobically to the same degree (Ehrlich, 1988; Ehrlich et al., 1991; Ehrlich, unpublished data). This raises a question about the differences in electron transport pathways between those organisms in which electron transport from acetate to MnO_2 is blocked by oxygen and those in which it is not. In at least some instances it may have to do with the nature of the manganese reductase. As discussed in the previous section, in marine pseudomonad BIII 88, which can reduce MnO_2 aerobically and anaerobically, the manganese reductase is postulated to act on Mn(III) produced by reaction between cell envelope bound Mn^{2+} and MnO_2 with which the cell surface is in contact. In *S. putrefaciens*, c-type cytochrome that occurs in the outer membrane of anaerobically but not aerobically grown cells is involved in MnO_2 reduction as it is in dissimilatory Fe(III) reduction (Myers and Myers, 1992, 1993, 2000). Indeed, Myers and Myers (2001) have presented strong evidence that cytochromes OcmA (Myers and Myers, 1997, 1998) and OcmB in the outer membrane of *S. putrefaciens* MR-1 are required for the reduction of MnO_2 but not for alternative soluble electron acceptors (see also discussion in Chap. 15). Direct evidence of electron transfer under anaerobic conditions between a putative 150 kD protein in the outer membrane of *S. putrefaciens* MR-1 and the surface of goethite (α-FeOOH) with which it is in contact has been reported by Lower et al. (2001). Cytochrome c_3 may be involved in MnO_2 reduction by *Desulfovibrio* spp., as it was shown to be in the reduction of

chromate (Lovley and Phillips, 1994b) and of uranium(VI) (Lovley et al., 1993a). A greater sensitivity of certain reduced cytochromes than that of Mn(II) to autoxidation may explain, at least in part, why some bacteria can reduce MnO_2 only anaerobically.

Bacterial Reduction of Mn(III)

Because Mn(III)-containing minerals like hausmannite exist, their possible function as terminal electron acceptors in bacterial respiration in nature needs to be considered. Complexed Mn(III) that can be abiologically formed in a reaction between MnO_2 and Mn^{2+} in the presence of a suitable ligand must also be considered as a potential terminal electron acceptor. Very limited evidence of such bacterial use of Mn(III) has been obtained to date. Gottfreund and Schweisfurth (1983) reported the reduction of Mn(III) pyrophosphate to Mn(II) by bacterial strain Red 16 using glucose, glycerol, or fructose as electron donor under aerobic conditions and glucose, fructose, glycerol, lactate, succinate, or acetate under anaerobic conditions. However, strain Red 16 could not grow on acetate either aerobically or anaerobically. The authors proposed that this organism produces Mn(III) in complexed form as a detectable intermediate in the reduction of Mn(IV) to Mn(II). Another study (Gottfreund et al., 1985) indicated the existence of bacteria that need not attack Mn(IV) but can produce a ligand that binds Mn(III). The Mn(III) was believed by the investigators to be derived by nonenzymatic reduction from Mn(IV). The bacteria then reduced the chelated Mn(III) to Mn(II). The authors suggested that Mn(III) is an intermediate in Mn(IV) reduction as well as in Mn(II) oxidation. However, whether Mn(III) is an obligatory intermediate in manganese oxidation or reduction has yet to be clearly established (see Ehrlich, 1988, 1993a, 1993b). The study of Mn(III) as an intermediate is fraught with difficulty because of the ease with which Mn(II) can react with some form of Mn(IV) in a disproportionation reaction to form Mn(III) in the presence of a ligand for Mn(III) such as pyrophosphate (e.g., Ehrlich, 1964).

More recently, Kostka et al. (1995) demonstrated the reduction of Mn(III) pyrophosphate by *S. putrefaciens* MR-1 using formate or lactate as electron donor. The reduction required anaerobic conditions and was inhibited by formaldehyde, tetrachlorosalicylanilide, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) but only slightly by nitrate and not at all by antimycin A. The alternative electron acceptor nitrate inhibited Mn(III) pyrophosphate reduction fully under aerobic conditions but only slightly under anaerobic conditions. Acetate, when it was the electron donor, was completely oxidized to CO_2. Energy derived from the reduction of Mn(III) pyrophosphate was able to drive protein synthesis. Abiotically, Mn(III)

pyrophosphate is reduced rapidly by Fe(II) and sulfide. Either of these reductants may be of microbial origin.

Considering insoluble forms of Mn(III), Larsen et al. (1998) demonstrated anaerobic manganite (MnOOH) reduction by a growing culture of *Shewanella putrefaciens* MR-4 in a lactate-containing medium. Reduction was optimal at pH 7 and 26°C. Cell contact was required for the reduction. It was inhibited by $HgCl_2$ (no concentration given) and by 0.1 mM carbonyl cyanide *m*-chloro-phenyl-hydrazone, and oxygen, but not by 0.2 mM cyanide or 0.05 mM 2-heptyl-4-hydroxyquinoline *N*-oxide.

Bacterial reduction of other Mn(III)-containing minerals seems not to have been reported so far but probably can occur.

Nonenzymatic Reduction of Manganese Oxides

Some bacteria and most of those fungi that reduce Mn(IV) oxides such as MnO_2 reduce them indirectly (nonenzymatically). A likely mechanism of reaction is the production of metabolic products that are strong enough reductants for Mn(IV) oxides. *Escherichia coli*, for instance, produces formic acid from glucose, which is capable of reacting nonenzymatically with MnO_2:

$$MnO_2 + HCOO^- + 3H^+ \rightarrow Mn^{2+} + CO_2 + 2H_2O \tag{16.13}$$

Pyruvate, which is a metabolic product of some bacteria can also react non-enzymatically with manganese oxides at acid pH (Stone, 1987). Sulfate reducers produce H_2S from sulfate, and *Shewanella putrefaciens* can produce H_2S and sulfite from thiosulfate and Fe^{2+} from Fe(III) in its respiratory processes. The resultant H_2S, sulfite, and Fe^{2+} can readily reduce some manganese oxides nonenzymatically (Burdige and Nealson, 1986; Mulder, 1972; Lovley and Phillips, 1988b; Myers and Nealson, 1988b; Nealson and Saffarini, 1994).

Many fungi produce oxalic acid, which can also reduce MnO_2 non-enzymatically (e.g., Stone, 1987):

$$MnO_2 + HOOCCOO^- + 3H^+ \rightarrow Mn^{2+} + 2CO_2 + 2H_2O \tag{16.14}$$

Because the electron transport mechanism in fungi, which are eukaryotic organisms, is located in mitochondrial membranes and not in the plasma membrane, as in prokaryotic cells, fungi cannot be expected to reduce MnO_2 enzymatically (Ehrlich, 1978a), except possibly by extracellular enzymes. MnO_2 reduction in acid solution under anoxic conditions by the reaction

$$MnO_2 + 2H^+ \rightarrow Mn^{2+} + 0.5O_2 + H_2O \tag{16.15}$$

is not very likely because of the requirement for a high energy of activation.

The fact that MnO_2 is reduced indirectly by fungi can be readily demonstrated on a glucose-containing agar medium into which MnO_2 has been

incorporated (e.g., Schweisfurth, 1968; Tortoriello, 1971). Fungal colonies growing on such a medium develop a halo (clear zone) around them in which MnO_2 has been dissolved (reduced). Because enzymes do not work across a spatial separation between them and their substrate, the formation of the halo can best be explained in one of two ways, which are not necessarily mutually exclusive. One way involves excretion by the fungus of a reducing compound, which then reacts with the MnO_2. The other way involves acidification of the medium by the fungus to a pH range at which a residual medium constituent like glucose reduces the MnO_2. The only exception would be the release of an enzyme by the fungus into the medium, which could then catalyze the reduction by a suitable electron donor. But the existence of such an enzyme has so far not been reported.

16.7 BIOACCUMULATION OF MANGANESE

Just as some microorganisms exist that accumulate ferric oxide, so do microorganisms that accumulate manganese oxides. In a number of instances, the same organism may accumulate either metal oxide or both. As with iron oxides, the organisms deposit manganese oxides on the surface of their cell envelope or on some structure surrounding it, such as a slime layer or a sheath, but not intracellularly (see reviews by Ghiorse, 1984; Ghiorse and Ehrlich, 1992; Ehrlich, 1999). In some instances the accumulation may represent the product of oxidation of Mn^{2+} by the organism; in others it may represent accumulation of manganese and/or iron oxides that were formed by other organisms or abiotically. Prokaryotes that accumulate manganese oxides include sheathed bacteria like *Leptothrix* (Fig. 15.8), budding and appendaged bacteria like *Metallogenium* (Fig. 16.8), *Pedomicrobium* (Fig. 16.9) (Ghiorse and Hirsch, 1979; Ghiorse and Ehrlich, 1992), *Hyphomicrobium* (Fig. 16.10), *Planctomyces* (Schmidt et al., 1981, 1982), *Caulococcus*, and *Kuznetsova*, bacteria with capsules like *Siderocapsa* (Fig. 15.11) and *Naumanniella*, and Mn^{2+}-oxidizing fungi (Fig. 16.11). As previously mentioned (see Sec. 16.5), the spores but not the vegetative cells of *Bacillus* SG-1 deposit manganese oxide on their surface. The formation of this oxide from Mn^{2+} is catalyzed by an enzyme in the exosporium.

Mineral deposition around microbial cells raises an interesting question that begs for an answer. Although bacteria with stalks or sheaths generally deposit manganese oxide only on these structures and can thus readily escape from such mineralizations by detaching from the stalk or escaping from the sheath, how do cells that become completely encased in such mineralization escape from it to multiply further? Are they permanently trapped in the mineralization and prevented from escaping it? It is possible that in nature, permanent entrapment in a solid coating of manganese and/or iron oxide of nonappendaged and

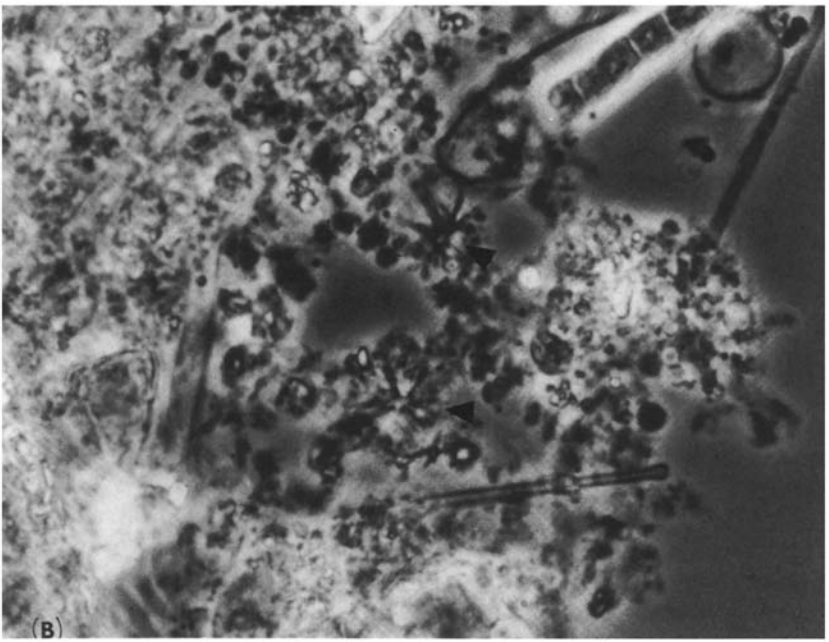


FIG. 16.8 *Metallogenium*. (A) Microaccretions of *Metallogenium* from Lake Ukshezero in a split peloscop after 50 days; growth in central part of microzone (×909). (From Perfil'ev and Gabe, 1965, with permission. (B) *Metallogenium* was filtered from Pluss See, Schleswig-Holstein, Germany (×2450). (Courtesy of W. C. Ghiorse and W.-D. Schmidt.)

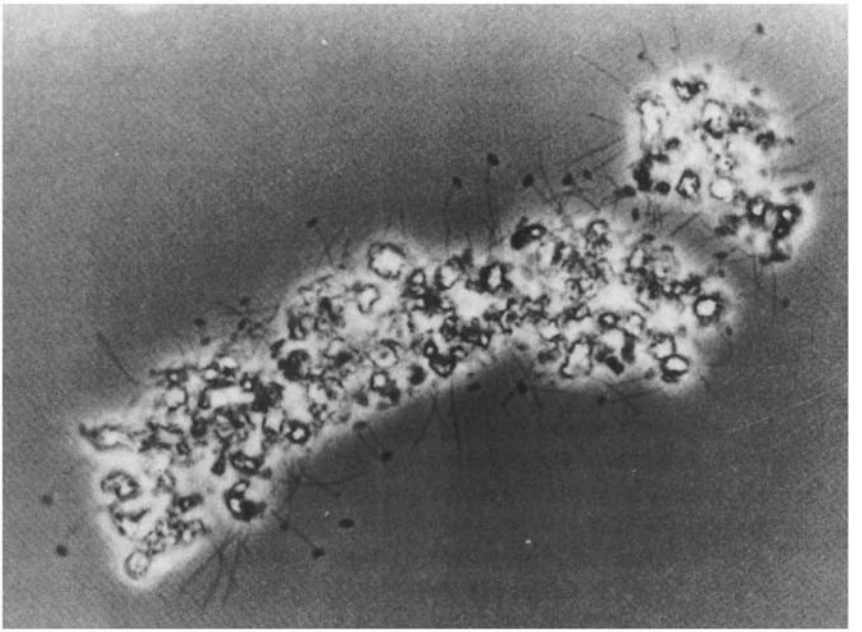


FIG. 16.9 *Pedomicrobium* in association with manganese oxide particles (×2800). (Courtesy of W. C. Ghiorse.)

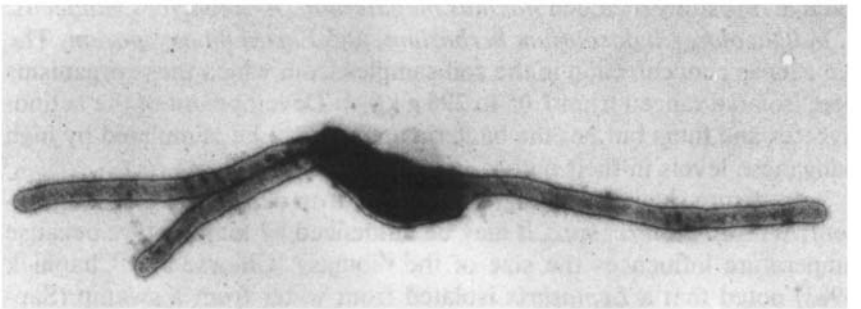


FIG. 16.10 Manganese-oxidizing *Hyphomicrobium* sp. isolated from a Baltic Sea iron-manganese crust (×15,600). (Courtesy of W. C. Ghiorse.)

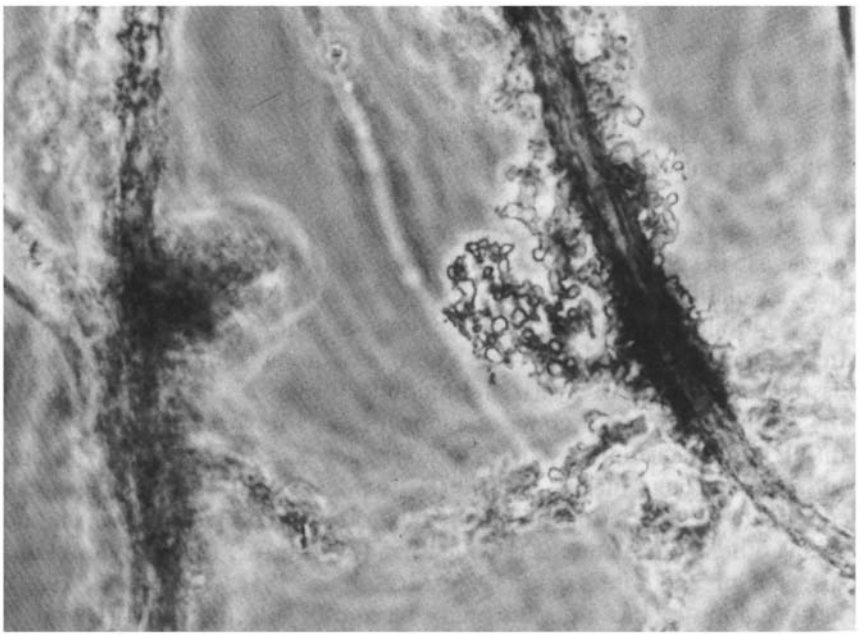


FIG. 16.11 Manganese oxide deposition on fungal hyphae (×900). (Courtesy of R. Schweisfurth.)

nonsheathed bacteria is preventable as long as their growth rate exceeds the mineral oxide deposition rate (see also Ehrlich, 1999).

Bioaccumulation of manganese in soil has been shown with certain bacteria, actinomycetes, and fungi isolated from soil of the Chiatura manganese biogeochemical province of the former Georgian S.S.R. They accumulated 0.04–0.3 mg of Mn per gram dry weight of biomass in Czapek's medium unsupplemented with manganese, and 9.2–101 mg g^{-1} biomass in Czapek's medium supplemented with 0.1% Mn (Letunova et al., 1978). The organisms analyzed in this study included *Bacillus megaterium*, *Actinomyces violaceus*, *A. indigocolor*, *Cladosporium herbarium*, and *Fusarium oxysporum*. The manganese concentration in the soil samples from which these organisms were isolated ranged from 1.05 to 296 g kg^{-1}. Development of the actinomycetes and fungi but not the bacteria appeared to be stimulated by high manganese levels in their native habitat.

From the standpoint of a natural environment, bacterial manganese and/or iron accumulation may be transient. With *Leptothrix* spp., it may be influenced by temperature because temperature influences the size of the biomass within its growth range. Ghiorse and Chapnik (1983) noted that a *Leptothrix* strain isolated

from water from a swamp (Sapsucker Woods, Ithaca, NY) grew optimally between 20 and 30°C and poorly at 4 and 35°C in the laboratory. This correlated with observations that *Leptothrix* and particulate iron and manganese were most abundant in the surface waters of the swamp when the temperature in the surface water was in the range of 20–30°C. Outside this temperature range, significantly less iron and manganese were found in these waters.

16.8 MICROBIAL MANGANESE DEPOSITION IN SOIL AND ON ROCKS

Soil

Manganese is an essential micronutrient for plants. Lack of sufficiently available manganese in soil can lead to manganese deficiency in plants, which may manifest itself in the form of grey-speck disease, for instance. Such lack of availability of manganese may be due to lack of its mobility in a soil and can be the result of activity of manganese-oxidizing microbes.

Microorganisms can have a profound effect on the distribution of manganese in soil. Oxidizers of Mn(II) will tend to immobilize it and make it less available or unavailable by converting it to an insoluble oxide. Mobile forms of manganese include Mn^{2+} ions, complexes of Mn^{2+}, especially complexes such as those of humic and tannic acids, complexes of Mn(III), and colloidal MnO_2. Immobile forms of manganese include Mn^{2+} adsorbed to clays or manganese or ferromanganese oxides, $Mn(OH)_2$, and insoluble salts of Mn(II) such as $MnCO_3$, $MnSiO_3$, and MnS, and various Mn(III) and Mn(IV) oxides. Oxidized manganese can accumulate as concretions in some soils, often accompanied by iron and various other trace elements (Drosdoff and Nikiforoff, 1940; Taylor et al., 1964; Taylor and McKenzie, 1966; McKenzie, 1967, 1970). The formation of these nodules can be microbially mediated (Drosdoff and Nikiforoff, 1940; Douka, 1977) (see also Sec. 16.5). The extent of insolubility of various immobile manganese compounds is governed by prevailing pH and E_h conditions (Collins and Buol, 1970). Reducing conditions at circumneutral or alkaline pH are especially favorable for the stability of insoluble manganous compounds, whereas oxidizing conditions at circumneutral or alkaline pH are especially favorable for the stability of Mn(III) and Mn(IV) oxides. Agriculturally, the most important forms of insoluble manganese include the oxides of Mn(IV) and mixed oxides such as $MnO \cdot MnO_2$ (also written Mn_2O_3) and $2MnO \cdot MnO_2$ or $MnO \cdot Mn_2O_3$ (also written Mn_3O_4). The stability of these oxides may also be affected by the presence of iron (Collins and Buol, 1970). Ferrous iron generated by Fe(III)-reducing bacteria can chemically reduce manganese(IV) oxides (Lovley and Phillips, 1988b; Myers and Nealson, 1988b).

Soil chemists have distinguished between the various forms of manganese in soil in an empirical fashion through the use of various extraction methods (see, e.g., Robinson, 1929; Sherman et al., 1942; Leeper, 1947; Reid and Miller, 1963; Bromfield and David, 1978). Thus, Sherman et al. (1942) measured, in successive steps, water-soluble manganese by extracting a soil sample with distilled water, then exchangeable manganese by extracting the residue with 1 N ammonium acetate at pH 7.0, and finally, easily reducible manganese by extracting the residue from the previous step with 1 N ammonium acetate containing 0.2% hydroquinone. Other investigators have used different extraction reagents to measure exchangeable and easily reducible manganese (e.g., Robinson, 1929; Leeper, 1947).

The soil percolation experiment by Mann and Quastel (1946) clearly showed the role that microbes can play in immobilizing manganese in soil. These investigators showed that when they continuously perfused nonsterile soil columns with 0.02 M $MnSO_4$ solution, the manganese was progressively retained in the columns by being transformed into oxide paralleled by a progressive disappearance of manganous manganese in the effluent. The oxidized state of the manganese was demonstrated by reacting it in situ with hydroxylamine reagent (a reducing agent). This reaction released manganese from the columns in soluble form. The basis for the oxidation of the perfused manganese in the columns was inferred to have been microbial activity because poisons such as chloretone, sodium iodoacetate, and sodium azide inhibited it.

Manganese oxidation by soil microbes can also be demonstrated in the laboratory by the method of Gerretsen (1937). It involves the preparation of Petri plates of agar mixed with unsterile soil. A central core is removed from the agar and replaced with a sterile sandy soil mixture containing 1% $MnSO_4$. As Mn^{2+} diffuses from the core into the agar, some developing bacteria and fungal colonies accumulate brown precipitate of manganese oxide in and around them as a result of enzymatic or nonenzymatic manganese oxidation, if the pH of the medium is held below 8 (Fig. 16.12). Controls in which the soil is antiseptically treated before adding it to the agar will not show evidence of manganese oxidation. This method was effectively used by Leeper and Swaby (1940) in demonstrating the presence of Mn^{2+}-oxidizing microbes in Australian soil.

Manganese(II) oxidation can also be studied in deep cultures of a soil agar mixture. Uren and Leeper (1978) studied manganese oxidation in deep cultures of soil agar mixtures (85 mL of 1% soil agar in a 150 mL jar) set up like a Gerretsen plate. The oxidation seemed to occur preferentially at reduced oxygen levels because oxidation was most intense 10–17 mm below the agar surface when air was passed over the surface. Above this oxidation zone, sparse manganese oxide was deposited only around bits of organic matter from the soil and around fungal hyphae. Raising the agar concentration in the soil medium to 2% caused the intense zone of oxidation to appear 4–11 mm below the surface. When the jar was

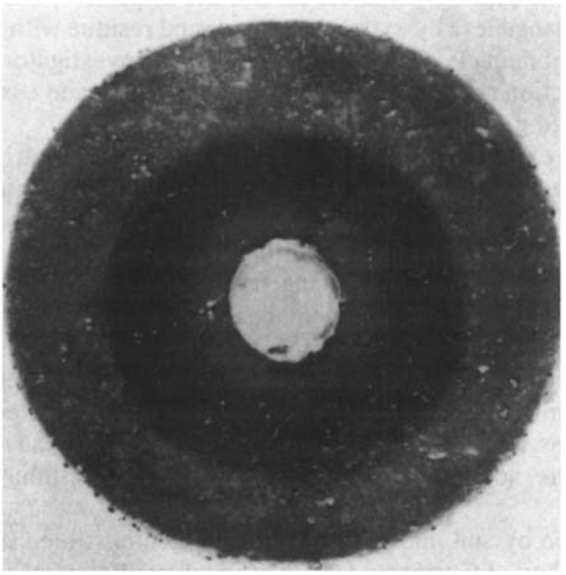


FIG. 16.12 Gerretsen plate by method modified by Leeper and Swaby (1940), showing manganese oxide deposition (dark halo) as a result of microbial growth around the central $MnSO_4$-containing agar plug. Initial pH 7.3, final pH 6.7. (From Leeper EW, Swaby RJ. The oxidation of manganous compounds by microorganisms in the soil, Soil Science 49:163–169. Copyright 1940 by Williams and Wilkins; with permission.)

sealed with an air space above the agar, an 8 mm thick oxidation zone developed from the surface downward. The investigators found that the factor that controlled the position of the zone of intense manganese oxidation in the soil column was available CO_2, because aeration at the surface with a mixture of 97% N_2, 2% O_2, and 1% CO_2 permitted development of an oxidation zone in the top 1 cm whereas a gas mixture of 98% N_2 and 2% O_2 did not. The observed oxidation was due to microbial activity because incorporation of azide to a final concentration of 1 mM into the soil agar inhibited manganese oxidation. Microbial manganese-oxidizing activity was noted even in soils as acid as pH 5.0 (Uren and Leeper, 1978; Sparrow and Uren, 1987).

In situ manganese oxidation by soil microbes was observed with pedoscopes (Perfil'ev and Gabe, 1965). This apparatus consists of one or more glass capillaries with optically flat sides for direct microscopic observation of the capillary content. Inserting a sterilized pedoscope into soil enables the development of soil microbes in the lumen of the capillaries under soil conditions. Periodic removal of the pedoscope and examination under a microscope permits visual assessment of the developmental progress of the organisms and the

deposition of manganese oxide, if any (see Chap. 7). Manganese-oxidizing and/or manganese-depositing organisms detected for the first time by use of a pedoscope include *Metallogenium personatum*, *Kuznetsova polymorpha*, *Caulococcus manganifer*, and others (Perfil'ev and Gabe, 1965, 1969).*

Application of the above techniques or others has provided ample evidence of the important role that microbes can play in immobilizing manganese in the soil by oxidizing it. The method of Gerretsen was adapted to show the role microbes play in manganese oxidation in Australian soil (Leeper and Swaby, 1940; Uren and Leeper, 1978; Sparrow and Uren, 1987) and in soils of South Sakhalin (former U.S.S.R.) (Ten Khak-mun, 1967). Other investigations have demonstrated the presence of manganese-oxidizing microbes (bacteria and/or fungi) in soils in various parts of the world using various methods. These include studies by Timonin (1950a, 1950b), Timonin et al. (1972), Bromfield and Skerman (1950), Bromfield (1956, 1978), Aristovskaya (1961), Aristovskaya and Parinkina (1963), Perfil'ev and Gabe (1965), Schweisfurth (1969, 1971), Van Veen (1973), and others. Among the bacteria identified as active agents of Mn(II) oxidation in these studies, *Arthrobacter* or *Corynebacterium*, *Pedomicrobium*, *Pseudomonas*, and *Metallogenium* were the most frequently mentioned, but a number of other unrelated genera were also identified as being active.

Rocks

Manganese oxides are sometimes found in thin, brown to black veneers, and iron oxides in orange veneers (each up to 100 μm thick) covering rock surfaces in some semiarid and arid regions of the world. In the brown to black coatings,

* The status of the genus *Metallogenium* is presently uncertain. Although isolated and cultured repeatedly in the former Soviet Union by a number of investigators (e.g., Perfil'ev and Gabe, 1965; Zavarzin, 1961, 1964; Mirchink et al., 1970; Dubinina, 1970, 1978b, 1984), living cultures that oxidize manganese have generally not been successfully isolated outside Russia. An exception is Herschel (1999), who isolated *Metallogenium* sp. (probably *M. personatum*) from a reservoir in Nordrhein-Westfalen, Germany, and cultured it in continuous culture in a chemostat. Structures resembling *Metallogenium* were collected by membrane filtration from waters of several lakes outside Russia, but these structures proved nonviable. Indeed, they did not contain any trace of nucleic acid, protein, or membrane lipid (Klaveness, 1977; Gregory et al., 1980; Schmidt and Overbeck, 1984; Maki et al., 1987). Structures interpreted as *Metallogenium symbioticum*, which is said to grow in obligate association with some fungi and bacteria (Zavarzin, 1961, 1964; Dubinina, 1984), may be fibrous manganese oxide produced nonenzymatically by the fungus (Schweisfurth, 1969; Schweisfurth and Hehn, 1972; Schmidt and Overbeck, 1984) as, for instance, in manganese oxidation by exopolymers produced by an arthroconidial anamorph of a basidiomycete (Emerson et al., 1989). It is possible that the starlike structures (arais) associated with *Metallogenium* coenobia (Balashova and Dubinina, 1989) are nonliving appendages from which the actual cell that gave rise to the appendages has become detached before or during sample collection. Like the stalks of *Gallionella*, the "appendages" may be structures impregnated with manganese oxide. A more recent observation of arais showing "secondary coccoid body formation" as part of a manganese-depositing biofilm on the inner walls of a water pipe lends support to this view (Sly et al., 1988).

manganese and/or iron oxides are major components (20–30%) along with clay (about 60%) and various trace elements (Potter and Rossman, 1977; Dorn, 1991). These manganese- or iron-rich coatings are known as **desert varnish** or *rock varnish*. Manganese-rich coatings have been detected on some rocks in the Sonoran and Mojave deserts (North America), Negev Desert (Middle East), the Gibson and Victoria deserts (Western Australia), and the Gobi Desert (Asia). Although they are very likely the result of microbial activity (Krumbein, 1969; Dorn and Oberlander, 1981; Taylor-George et al., 1983; Hungate et al., 1987), this view is not universally shared. Krumbein (1969) and Krumbein and Jens (1981) implicated cyanobacteria and fungi in the formation of desert varnish in the Negev Desert, whereas Dorn and Oberlander implicated *Pedomicrobium*- and *Metallogenium*-like bacteria in desert varnish from Desert Valley in the Mojave Desert. The latter investigators produced desert varnish–like deposits in the laboratory with their bacterial isolates. Dorn (1991) is of the opinion that the following conditions favor bacterial participation in manganese-rich varnish formation: (1) moist rock surfaces, (2) rock surfaces of low nutrient content that favor bacterial manganese oxidation by mixotrophs, and (3) rock surfaces exhibiting circumneutral pH.

Adams et al. (1992) suggested that in the formation of iron-rich varnish, siderophores, produced by bacteria on rock surfaces where the varnish forms, mobilize the ferric iron from windborne dust or rock surfaces. The mobilized iron is then concentrated as iron oxide or oxyhydroxide on the cell walls of bacteria on the rock surfaces. Upon the death of the iron-coated bacteria, the iron oxide becomes part of the varnish.

Microscopic examination of desert varnish from the Sonoran Desert revealed the presence of fungi (dematiaceous hyphomycetes) and bacteria. Manganese oxidizers in samples from this source included *Arthrobacter*, *Micrococcus*, *Bacillus*, *Pedomicrobium*, and possibly *Geodermatophilus* (Taylor-George et al., 1983). Active respiration but little CO_2 fixation was detected in the varnish. The respiration was attributed mainly to the fungi. The absence of significant CO_2 fixation indicated an absence of photosynthetic, i.e., cyanobacterial or algal, activity. Varnish formation on Sonoran Desert rocks has been postulated to be the result of formation of fungal microcolonies on rock surfaces, which trap windborne clay and other mineral particles. Upon death of the fungi, intermittent, moisture-dependent weathering of minerals is thought to lead to microbial and/or abiotic concentration of mobilized manganese through oxidation. Dead fungal biomass could provide nutrients to manganese-accumulating bacteria at this stage. Further development of fungal microcolonies and their coalescence was postulated to lead ultimately to the formation of continuous films of varnish. Besides contributing to rock weathering, the fungal mycelium in this model seems to serve as an "anchor" for Mn oxide formed at least in part by bacteria (Taylor-George et al., 1983). The MnO_2 in the varnish may act as a screen against ultraviolet radiation and may thus serve a protective function

(Taylor-George et al., 1983). Examination of the bacterial flora of desert varnish from the Negev Desert yielded a range of bacterial isolates similar but not identical to that from varnish from the Sonoran Desert. Of 79 bacterial isolates, 74 oxidized manganese under laboratory conditions. They were assigned to the genera *Bacillus*, *Geodermatophilus*, *Arthrobacter*, and *Micrococcus* (Hungate et al., 1987). The prevalence of manganese oxidizers in desert varnish samples from geographically very distinct desert sites further supports the idea that they play a role in its formation (Hungate et al., 1987).

Ores

It has been suggested that some sedimentary manganese ore deposits are of biogenic origin. This is based in part on the observation of structures in the ore that have been identified as microfossils by the discoverers. Examination of manganese ore from the Groote Eylandt deposit in Australia revealed the presence of algal (cyanobacterial?) stromatolite structures, cyanobacterial oncolites, coccoid microfossils, and microfossils enclosed in metacolloidal oxides of manganese (Ostwald, 1981). In this deposit, the microbes are viewed as being the main cause of manganese oxide formation and accretion, with subsequent nonbiological diagenetic changes leading to the ultimate form of the deposit. Some Precambrian and a Cretaceous-Paleocene manganese deposit (Seical deposit on the island of Timor) have been attributed to the activity of *Metallogenium* because coenobial structures resembling modern forms of this organism as described by Russian investigators have been found by microscopic study of sections of the respective ores (Crerar et al., 1979). Similar observations were made by Shternberg (1967) on samples of the Oligocene Chiatura manganese deposit and the Paleozoic Tetri-Tsarko manganese deposit.

16.9 MICROBIAL MANGANESE DEPOSITION IN FRESHWATER ENVIRONMENTS

The first reports on manganese-oxidizing microorganisms in freshwater environments appeared at the beginning of the twentieth century. They include those of Neufeld (1904), Molisch (1910), and Lieske (1919) as cited by Moese and Brantner (1966), Thiel (1925), von Wolzogen-Kühr (1927), Zappfe (1931), and Sartory and Meyer (1947). The organisms found by these investigators were usually detected in sediments, organic debris, or manganiferous crusts. More recent investigations dealt with microbial manganese oxidation in springs, lakes, and water distribution systems.

Bacterial Manganese Oxidation in Springs

Precipitation of manganese hydroxide (presumably hydrous manganese oxide), which upon aging became transformed into pyrolusite and birnessite in a mineral spring near Komaga-dake on the island of Hokkaido, Japan, was attributed to the activity of manganese-oxidizing bacteria, including sheathed bacteria (Hariya and Kikuchi, 1964). The bacteria were detected by filtration of water from the spring through ordinary filter paper and membrane filters and by incubating samples of water in the laboratory. The water contained (in mg L^{-1}): Mn^{2+}, 4.75; K^{+}, 16; Na^{+}, 128; Ca^{2+}, 101; Mg^{2+}, 52; Cl^{-} 156.2; $SO_4{}^{2-}$, 481; $HCO_3{}^{-}$ and CO_2, 11; but only traces of Fe^{2+} or Fe^{3+}. The temperature of the water was 23°C, and its pH was 6.8. During incubation of water samples in the laboratory, manganese precipitated progressively between 20 and 50 days accompanied by a fall in pH and a gradual rise in E_h after 20 days and an abrupt rise after 45 days. Only 1.18 mg of dissolved Mn^{2+} per liter was left after 50 days.

Another instance of bacterial manganese oxidation associated with a spring was reported from Squalicum Creek Valley near Bellingham, Washington (Mustoe, 1981). In this example, a black soil surrounded the spring over an area 5 m × 25 m and to a depth of 30 cm. It contained 43% MnO_2 and 20–30% iron oxide (classified as Fe_2O_3). Two pseudomonad strains that oxidized Mn^{2+} and Fe^{2+} when growing on a medium containing soil organic matter were isolated from it. The oxides were deposited extracellularly. The isolates were considered to be a cause of manganese and iron deposition in soil.

Still another instance of bacterial manganese oxidation in spring water and sediment was observed near Ein Feshkha on the western shore of the Dead Sea, Israel (Ehrlich and Zapkin, 1985). Gram-positive, spore-forming rods and gram-negative rods were isolated from water and sediment samples from drainage channels, which oxidized Mn^{2+} enzymatically. They were of Group I type (see Sec. 16.5). It was postulated that at least some of the manganese oxide formed by the bacteria in the spring water was carried to the Dead Sea and became incorporated as laminations in calcareous crusts found along the shore of the lake.

Bacterial Manganese Oxidation in Lakes

Evidence of bacterial manganese oxidation has been observed in Lake Punnus-Yarvi on the Karelian Peninsula in the former U.S.S.R. (Sokolova-Dubinina and Deryugina, 1967a, 1967b). The lake is of glacial origin, oligotrophic, and slightly stratified thermally. It is 7 km long, up to 1.5 km wide, and up to 14 m deep. It contains significant amounts of dissolved manganese (0.02–1.4 mg L^{-1}) and iron (0.7–1.8 mg L^{-1}) only in its deeper waters. The lake is fed by two rivers and 24 streams, which drain surrounding swamps. The manganese in the lake is supplied by surface- and groundwater drainage containing 0.2–0.8 mg Mn L^{-1} and 0.4–

2 mg Fe L^{-1}. Most of the iron and manganese in the lake are deposited on the northwestern banks of Punnus-Ioki Bay situated at the outflow from the lake into the Punnus-Ioki River in a deposit 5–7 cm thick at a water depth of up to 5–7 m. The deposit includes hydrogoethite ($nFeO \cdot nH_2O$), wad ($MnO_2 \cdot nH_2O$), and psilomelane ($mMnO \cdot MnO_2$). The iron content of the deposit ranges from 18% to 60% and the manganese content from 10% to 58%. The deposit also includes 5–16% SiO_2 and Al, Ba, and Mg in amounts ranging from 0.3% to 0.7%. The bacterium held responsible for manganese oxidation was *Metallogenium*, which was found in all parts of the lake.

Manganese deposition was demonstrated in the laboratory by incubating sediment samples at 8°C for several months. Dark brown compact spots of manganese oxide were then detected, which revealed no characteristic bacterial structures by examination with a light microscope but did show by electron microscopy the presence of *Metallogenium* that closely resembled *Metallogenium* fossils in Chiatura manganese ore (Dubinina and Deryugina, 1971). *Metallogenium* was cultured from lake samples on manganese acetate agar. No *Metallogenium* cultures were obtained from sediment samples from parts of the lake where no microconcretions of manganese oxide were found. *Metallogenium* development was also studied in peloscopes, which showed progressive encrustation with manganese oxide. Manganese deposition in the lake occurred in a redox potential range of +435 to +720 mV and a pH range of 6.3–7.1. It was concluded from data gathered by other investigators that ore formation may begin at an E_h as low as +230 mV and at a pH of 6.5. At Mn^{2+} concentrations below 10 mg L^{-1}, autoxidation was not considered likely. Manganese oxide deposition attributed to *Metallogenium* activity was also noted in some other Karelian lakes where a steady supply of dissolved manganese occurred, redox conditions were favorable, and bacterial reducing processes did not occur (Sokolova-Dubinina and Deryugina, 1968). More recently, *Metallogenium* was reported to occur in sediment samples from Lake Geneva (Lac Leman) (Jaquet et al., 1982) and was implicated in manganese-oxidizing activity in Lake Constance (Stabel and Kleiner, 1983).

In Lake Oneida, near Syracuse, New York, manganese deposition has been studied for many years because of the occurrence of ferromanganese concretions on the sediment surface of shallow, well-oxygenated central areas of the lake (Fig. 16.13). The lake has a surface area of 207 km^2 and varies in depth, its average depth being 6.8 m and its maximum depth 16.8 m (Dean and Ghosh, 1978). It never exhibits seasonal thermal stratification. Wind agitation keeps it well aerated to all depths when it is not frozen, although deeper water may have oxygen concentrations only 50% of saturation (Dean and Ghosh, 1978). The ferromanganese concretions appear as crusts around rocks at the edge of shoals where the water is less than 4.3 m in depth (Dean, 1970), but some have also been recovered from hard sediments at depths of 8 m (Chapnick et al., 1982). The crust may take on a saucer shape, concave upward (Dean, 1970). Many exhibit coarse concentric

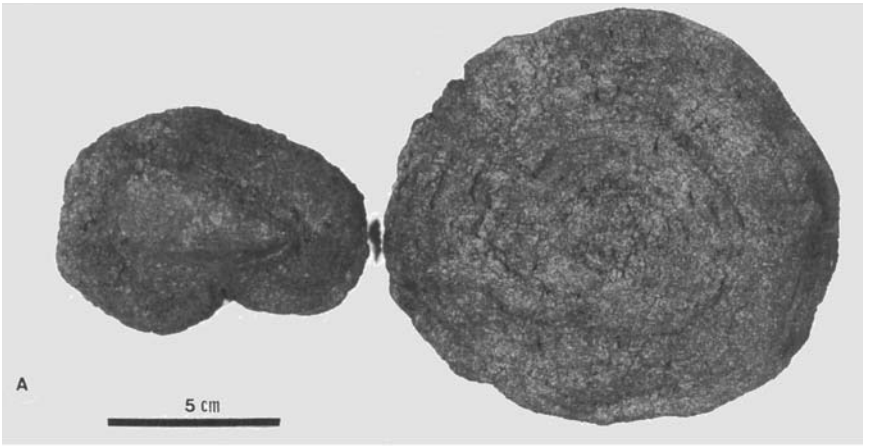
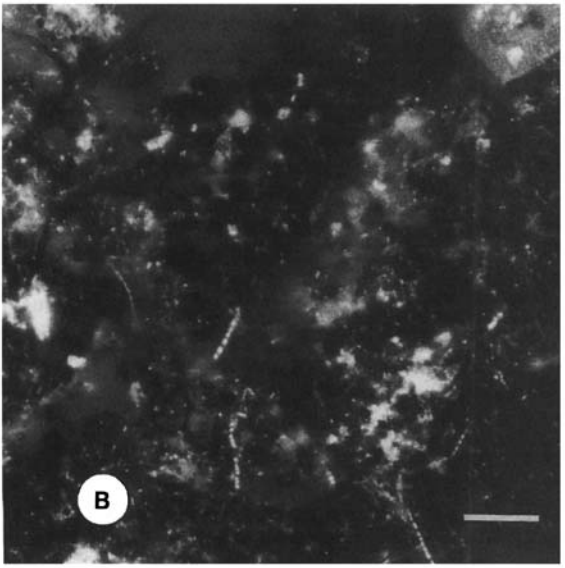
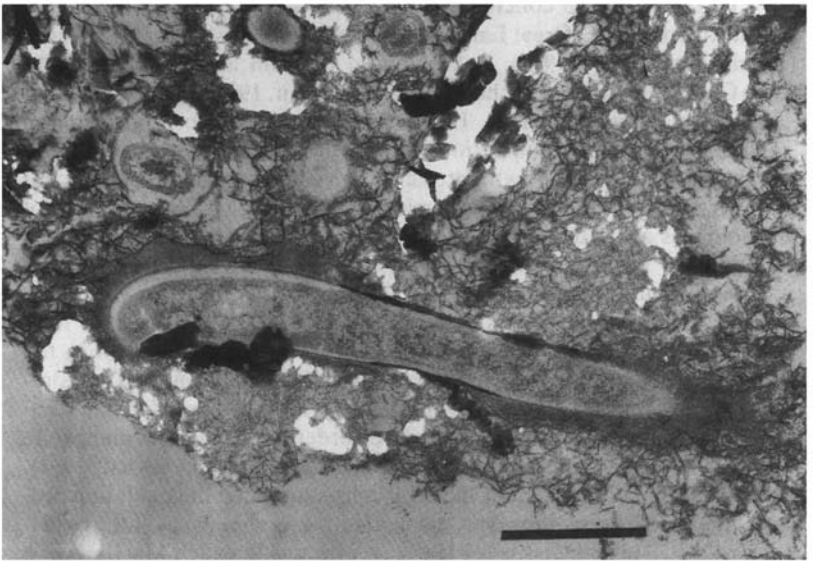


FIG. 16.13 Ferromanganese concretions from the "nodule-rich" area in Lake Oneida, central New York State. (A) Two concretions: the one on the right is typical of the flat "pancake" variety, the one on the left is a rock with 2–3 cm of ferromanganese oxide crust. (B) Epifluorescent light photomicrograph of a portion of the surface of the concretion on the left in (A) stained with 0.01% acridine orange. The sample was removed from the surface and then viewed under ultraviolet epi-illumination. The brightly fluorescent filamentous and coccoid bacteria are present in the ferromanganese-mineralized biofilm on the surface. Scale bar = 10 μm. (C) Transmission electron photomicrograph of an ultrathin section of a piece of material from a concretion similar to those shown in (A). The sample was fixed in glutaraldeyde followed by osmium tetroxide and uranyl acetate, dehydrated in ethanol, and embedded in epoxyresin. A portion of a filamentous bacterial cell approximately 0.6 μm in diameter is surrounded by a dark-colored complex composed of polymer-ferromanganese oxide. Note the smaller encrusted coccoid cell in upper left surrounded by a clear zone produced by shrinkage during preparation for electron microscopy. The wispy dendritic black material is Fe/Mn oxide. Scale bar = 1 μm. (From Ghiorse and Ehrlich 1992; with permission.)

banding of alternating zones rich in manganese and rich in iron (Moore, 1981). At other times the concretions have been described as flattened, disc-shaped structures. Their rate of growth as determined by natural radioisotope analysis has been estimated to vary between >1 mm per 100 years at some periods and no growth at others (Moore et al., 1980).

The origin of the ferromanganese concretions in Lake Oneida has been a puzzle. Hypotheses of abiogenesis (e.g., Ghosh, 1975) and biogenesis (e.g., Gillette, 1961; Dean, 1970; Dean and Ghosh, 1978; Dean and Greeson, 1979; Chapnick et al., 1982) have been offered at various times. Gillette (1961) and





(C)

FIG. 16.13 (*continued*)

Chapnick et al. (1982) favor a direct bacterial role, whereas Dean (1970) and Dean and Ghosh (1978) favor an indirect role played by algae in the lake. Gillette (1961) recognized bacterial cells in pulverized concretions by microscopic examination and showed that some of the isolates precipitated iron. He speculated that bacterial deposition of iron and manganese in the lake could be the result of degradation of organic complexes but also suggested that iron may be precipitated by autoxidation with oxygen generated in the photosynthesis of algae. Dean and Ghosh (1978) and Dean and Greeson (1979) favor a primary role for cyanobacteria and algae as producers of chelators for iron and manganese and accumulators of the chelates. These notions are supported by recent observations that cyanobacteria in the lake belonging to the genus *Microcystis* sp. generate microenvironments with strongly alkaline conditions (pH >9) in their mats as a result of photosynthetic activity (Richardson et al., 1988) [see Eqs. (15.27) and (15.28) in Chap. 15 for a chemical explanation]. Such alkaline conditions promote autoxidation of Mn^{2+}. The oxides become entrapped in the mats. Upon death, the cyanobacterial and algal biomass is believed to carry the oxidized iron and manganese to the lake bottom to be released on the decay of the biomass and somehow incorporated into concretions. Chapnick et al. (1982) demonstrated that bacteria in the bottom water of the lake are able to catalyze the oxidation of the Mn^{2+} dissolved in it. They also noted some binding of Mn^{2+} without oxidation. Analysis of a manganese budget for the lake supports the notion that most of the dissolved manganese (95%) becomes incorporated into the nodules. Chapnick et al. suggested that cyanobacteria and algae help in transporting the manganese from the suface waters to the nodule-forming regions where bacteria participate in the oxidation of Mn^{2+} in the bottom waters to an as yet undetermined extent. Bottom water movement carries nodules or fragments of nodules to the surface of deeper anoxic sediments, where they are buried and undergo reduction with remobilization of manganese. *Shewanella* (formerly *Alternaria*) *putrefaciens* is one organism found in this sediment that has been demonstrated in laboratory experiments to be an effective reducer of Mn(IV) oxide (Myers and Nealson, 1988a). Deep water circulation reintroduces the dissolved manganese into the water column, and the cycle is repeated. Manganese that is lost in the lake outflow (50 tons yr^{-1}) is more than replaced by manganese influx (75 tons yr^{-1}) into the lake (Dean et al., 1981).

Another lake in which ferromanganese concretions have been found is Lake Charlotte, Nova Scotia, first studied by Kindle (1932) and later by Harriss and Troup (1970). The concretions occur in a shallow region of the lake that Kindle (1935) named Concretion Cove. It receives runoff from soil rich in metals and organics. Kindle (1932) thought that an indirect mechanism was operating in which removal of CO_2 from the lake water through photosynthesis by diatoms caused a rise in pH that promoted autoxidation of Mn^{2+} to MnO_2. Later investigations suggested more direct bacterial involvement (Kepkay, 1985a,

1985b). Using a special device called a peeper (Burdige and Kepkay, 1983) emplaced into sediment in Concretion Cove, Kepkay (1985a, 1985b) demonstrated bacterially dependent oxidation of Mn(II) in the dark, i.e., without direct participation of algal photosynthesis. He also demonstrated the binding of nickel and, to a lesser extent, copper that was enhanced by microbial oxidation of manganese. In experiments in which filter membranes with 0.2 μm pore size were submerged in Lake Charlotte water, a succession of bacterial cell types were observed: cocci developed into rods, suggesting *Arthrobacter*. The coccus-to-rod transformation coincided with a peak in oxygen consumption. CO_2 fixation was detectable as the rods started to bind Mn and Fe to their cell suface. If this metal binding involved, at least in part, oxidation, especially that of manganese, autotrophic growth at the expense of manganese could have been taking place at that point (Kepkay et al., 1986).

Sediment samples from the experimental site in Lake Charlotte where concretions are found revealed the presence of microscopic precipitates of manganese and iron (Kepkay, 1985a, 1985b). How these precipitates are related to the concretions has yet to be elucidated. In places where macrophytes (*Eriocaulon sepangulare*) grew in the Concretion Cove area, bacterial manganese oxidation resulted in the formation of finely dispersed oxide within the sediments. In places where macrophytes were absent, ferromanganese concretions were noted at the sediment surface. The macrophyte root system appeared to influence manganese oxidation by somehow promoting downward movement of manganous manganese in the sediments. Manganese oxidation was most active 1–3 cm below the sediment surface where the macrophytes grew but also occurred at the sediment surface where macrophytes were absent (Kepkay, 1985c).

Ferromanganese concretions have been reported in still other lakes in North America. Examples include Great Lake, Nova Scotia, and Mosque Lake, Ontario (Harriss and Troup, 1970); Lake Ontario (Cronan and Thomas, 1970); Lake George, New York (Schöttle and Friedman, 1971); and Lake Michigan (Rossman and Callender, 1968). Typical compositions of the concretions are listed in Table 16.5. In general, concretions form in areas of lakes where the sedimentation rate is low. Growth rates of concretions may be very slow, as in Lake Ontrario ($0.015\,mm\,yr^{-1}$), or more rapid, as in Mosque Lake ($1.5\,mm\,yr^{-1}$). Not all geologists have been convinced that microbes play a role in formation of the concretions. Varentsov (1972), for instance, explained the formation of such nodules in Eningi-Lampi Lake, Karelia, Russia, in terms of chemosorption and autocatalytic action.

One instance of possible transient manganite (γ-MnOOH) formation in an artificial lake was reported by Greene and Madgwick (1991). The lake was created behind a tailings dam in the mining operation of the Mary Kathleen Mine, North Queensland, Australia. In it bacteria occur in association with the microalga *Chlamydomonas acidophilus* that oxidize Mn^{2+} to form disordered γ-MnO_2

TABLE 16.5 Composition of Some Lake Ores (Values in Percent)

Mn	Fe	Si	Al	Source	Reference
36.08 (MnO_2)	13.74 (Fe_2O_3), 7.70 (FeO)	12.75 (SiO_2)	12.50 (Al_2O_3)	Ship Harbour Lake	Kindle (1932)
13.4–15.4 (Mn)	19.5–27.5 (Fe)	4–10 (Si)	0.7–0.95 (Al)	Oneida Lake	Dean (1970)
31.7–35.9 (Mn)	14.2–20.9	—	—	Great Lake	Harriss and Troup (1970)
15.7 (Mn)	39.8–40.2 (Fe)	—	—	Mosque Lake	Harriss and Troup (1970)
17.0 (Mn)	20.6 (Fe)	—	—	Lake Ontario	Cronan and Thomas (1970)
3.57 (Mn)	33.2	—	—	Lake George	Schöttle and Friedman (1971)
0.89–22.2 (MnO)	1.34–60.8 (FeO)	—	—	Lake Michigan	Rossman and Callender (1968)

via manganite under laboratory conditions (Greene and Madgwick, 1991). An unidentified component of the algal cells stimulated the oxidation process. Intact cells, however, were most effective as stimulants of the oxidation process. This process is evidently an example in which Mn(III) oxide appears to be an intermediate in Mn(IV) oxide formation.

Bacterial Manganese Oxidation in Water Distribution Systems

In 1962, a case of microbial manganese precipitation in a water pipeline connecting a reservoir with a filtration plant of the waterworks in the city of Trier, Germany, was reported (Schweisfurth and Mertes, 1962). The accumulation of precipitate in the pipes caused a loss of water pressure in the line. The sediment in the pipe had a dark brown to black coloration and was rich in manganese but relatively poor in iron content. Microscopic examination of the sediment revealed the presence of cocci and rods after removal of MnO_2 with 10% oxalic acid solution. Sheathed bacteria were found only on the rubber seams, and then always in association with other bacteria. Evidence of fungal mycelia and streptomycetes was also found. In culture experiments, only gram-negative rods and fungi were detected. Chemical examination of the reservoir revealed that the manganese concentration in the bottom water was between 0.25 and $0.5\,mg\,L^{-1}$ during most of 1960, except in September and October, when it ran as high as $6\,mg\,L^{-1}$. The peak in manganese concentration in the bottom waters was correlated with water temperature, which reached its peak at about the same time as the manganese concentration in the water. The two feed streams into the reservoir did not contribute large amounts of manganese, only about $0.05\,mg\,L^{-1}$. The major source of the manganese could have been the manganiferous minerals of the reservoir basin and surrounding watershed.

Similar observations were made in some pipelines connecting water reservoirs with hydroelectric plants in Tasmania, Australia (Tyler and Marshall, 1967a). In this instance, pipelines from Lake King William were found to have heavy deposits of manganese oxide, whereas those from Great Lake did not. The deposits in pipes leading from Lake King William accumulated to a maximum thickness of 7 mm in 6–12 months. The manganic oxide deposition process was reproduced in the laboratory in a recirculatory apparatus (Tyler and Marshall, 1967a). With water from Lake King William, a deposition of a brown manganic oxide formed at the edge of coverslips after 24 hr and for 6 days thereafter. Subsequent addition of $MnSO_4$ to the water caused further deposition after 6 days. By contrast, only traces of deposit developed with Great Lake water in similar experiments. The difference in manganese deposition from the two lake waters was explained in terms of the difference in their manganese content. Lake King Williams water contained 0.01–0.07 ppm of manganese, whereas Great

Lake water contained only 0.001–0.013 ppm. In the laboratory, inoculation of Great Lake water with some Lake King William water did not promote the oxidation unless $MnSO_4$ was also added. It was also found that if Lake King William water was autoclaved or was treated with azide (10^{-3} M final concentration), manganic oxide deposition was prevented, suggesting the participation of a biological agent in the reaction. Inoculation of autoclaved Lake King William water with untreated water caused resumption of the oxidation. The dominant organism involved in the oxidation appeared to be one identified as *Hyphomicrobium* sp. Sheathed bacteria and fungi and possibly *Metallogenium symbioticum* were also encountered in platings from the pipeline deposit, but only at low dilutions. It was not resolved whether *Hyphomicrobium* sp. oxidized Mn^{2+} enzymatically or nonenzymatically (Tyler and Marshall, 1967b). After the publication of the original studies of manganese oxide pipeline deposits in Tasmania, Tyler (1970) found *Hyphomicrobium* in pipeline deposits in other parts of the world. It was not always the only manganese-oxidizing organism present, however.

In a seasonal study of North Pine Dam, a body of freshwater located 25 km northwest of Brisbane, Australia, Johnson et al. (1995) noted that microbial manganese oxidation prevailed in the lower epilimnion between November and May (Australian summer). Abiotic manganese oxidation became more noticeable in late summer and autumn, when it represented up to 25% of the total. Microbial oxidation required a minimum temperature of 19°C and was optimal at 30°C. The oxidation state of the oxidized manganese ranged from 2.55 to 3.25 in the epilimnion and was 3.59 in a hypolimnion sample after a few days of aerobic incubation. It was highest when microbial activity was greatest.

Micronodule formation in biofilms formed by *Pseudomonas* spp. on the surface of polyvinyl chloride (PVC) and high-density polyethylene (HDPE) pipe material after 2 weeks of incubation was reported by Murdoch and Smith (1999). The nodules had an average diameter of 10 μm. Most featured a ~2 μm hole at their center. They were rich in manganese. The role of the microbes in the micronodule-forming process remains to be elucidated.

16.10 MICROBIAL MANGANESE DEPOSITION IN MARINE ENVIRONMENTS

Manganese-oxidizing bacteria have been detected in various parts of the marine environment, including the water column and surface sediments in estuaries, continental shelves and slopes, and abyssal depths. They appear to play an integral part in the manganese cycle in the sea.

Manganese is unevenly distributed in the marine environment. It occurs in greater quantities on and in sediments than in seawater and in greater quantities in

TABLE 16.6 Manganese Budget for the Pacific Ocean

Total Mn (as MnO) in sediments	1.4×10^{15} tons
Total Mn (as MnO) in nodules	3.1×10^{11} tons (170 times)[a]
Total Mn (as MnO) in seawater	1.8×10^{9} tons
Total Mn (as MnO) in biomass	1×10^{7} tons (0.0055 times)[a]

[a] Relative to manganese in seawater.
Source: Based on data from Poldervaart (1955), Mero (1962), and Bowen (1966).

seawater than in biomass (see Table 16.6 for Mn distribution in the Pacific Ocean). In some parts of the oceans, a significant portion of manganese at the sediment interface at abyssal depths is concentrated in ferromanganese concretions (nodules) and crusts. The concentration of manganese in surface seawater from the Pacific Ocean has been reported to fall in the range of 0.3–3.0 nmol kg^{-1} (16.4–164.8 ng kg^{-1}) (Klinkhammer and Bender, 1980; Landing and Bruland, 1980). The concentration in bottom water is generally less than that in surface water. Klinkhammer and Bender (1980) found it to be one-fourth or less at some stations in the Pacific Ocean. Manganese concentration in surface waters over the continental slope near the mouth of major rivers such as the Columbia River on the coast of the state of Washington may be as high as 5.24 nmol kg^{-1} (164.8 ng kg^{-1}) (Jones and Murray, 1985). Exceptionally high manganese concentrations occur around active hydrothermal vents on mid-ocean spreading centers. Manganese concentrations in hydrothermal solution as high as 1002 μmol kg^{-1} (55.05 mg kg^{-1}) have been reported (Von Damm et al., 1985). Although diluted as much as 8500 times within tens of meters from a vent source when the hydrothermal solution mixes with bottom water, elevated manganese concentrations may be encountered in plumes extending 1 km or more from the vent source (Baker and Massoth, 1986), and in some instances for hundreds of kilometers.

The dominant oxidation state of manganese in seawater is +2 despite the alkaline pH of seawater (7.5–8.3) (Park, 1968) and its E_h of +430 mV (ZoBell, 1946). The stability of the divalent manganese in seawater is attributable to its complexation by the abundant chloride ions (Goldberg and Arrhenius, 1958), by sulfate and bicarbonate ions (Hem, 1963), and by organic substances such as amino acids (Graham, 1959). The oxidation state of manganese in marine sediments and ferromanganese concretions is mainly +4 based on samples taken in the Pacific Ocean north of the equator and in the Central Indian Ocean (Kalhorn and Emerson, 1984; Murray et al., 1984; Pattan and Mudholkar, 1990). A primary source of manganese in the oceans is that injected by hydrothermal solutions at vents on mid-ocean spreading centers. Magma and

volcanic exhalations from submarine volcanoes also make a contribution. Other contributions are from continental runoff and from aeolian sources (windblown dust).

Manganese at the low concentrations found in most of the seawater is important biologically as a micronutrient but is too dilute to serve as energy source without prior concentration. The manganese assimilated by marine organisms can be viewed as transiently immobilized but is returned to the available manganese pool upon the death of the organisms.

Microbial Manganese Oxidation in Bays, Estuaries, Inlets, *etc.*

Thiel (1925) detected manganese-oxidizing bacteria in marine mud collected in Woods Hole, Massachusetts. He also found that in anaerobic culture, sulfate-reducing bacteria precipitated $MnCO_3$. Krumbein (1971) reported the presence of heterotrophic bacteria and fungi in the Bay of Biscay and in the Heligoland Bight (North Sea) by culturing sediment samples collected at these locations. In the Bay of Biscay, the organisms were present in all samples collected in the uppermost millimeters of sediment at water depths of 13–180 m, but few (in only one of several samples taken) between 280 and 540 m. In plate counts from sediment and water samples from the estuary of the River Tamar and the English Channel, Vojak et al. (1985a) found that 11–85% of the total bacterial population formed colonies representing manganese-oxidizing bacteria. The plating was done on an $MnSO_4$-containing culture medium prepared in seawater (3% sea salt) with peptone and yeast extract. Vojak et al. (1985a) identified the manganese-oxidizing colonies by applying berbelin blue reagent (Krumbein and Altmann, 1973), which is turned blue by oxidized manganese. No obvious correlation between total number of bacteria, proportion of manganese oxidizers, and particulate load or salinity was noted, nor were any seasonal trends with regard to distribution of manganese oxidizers observed. Manganese oxidation rates in water from Tamar Estuary amended with $2\,mg\,Mn^{2+}\,L^{-1}$ were $3.32\,\mu g\,L^{-1}\,hr^{-1}$ in freshwater containing $30\,mL\,L^{-1}$ suspended matter and $0.7\,\mu g\,L^{-1}\,hr^{-1}$ in saline water (32‰ salinity) with the same amount of suspended matter. The rate of manganese oxidation was proportional to particulate load up to $100\,mg\,L^{-1}$. Oxidation was depressed in the presence of metabolic inhibitors such as chloramphenicol ($100\,\mu g\,mL^{-1}$). The oxidation had a temperature optimum of 30°C, which was above the in situ temperature (13.5°C) when the water sample was taken (Vojak et al., 1985b).

Saanich Inlet, on the southeast side of Vancouver Island, British Columbia, Canada, presents an interesting natural setting in which to study bacterial Mn^{2+} oxidation. As described by Anderson and Devol (1973) and Richards (1965) (see also Tebo and Emerson, 1985), it is a fjord having a maximum water depth of

220 m and features a sill at its mouth at a depth of 70 m. Water behind the sill develops a chemocline in late winter and summer, becoming anoxic below 130 m. The anoxic condition prevails for about 6 months, after which it is displaced by dense oxygenated water pushed over the sill as a result of strong coastal upwelling. Bacterial Mn^{2+}-oxidizing activity was measured just above the O_2/H_2S interface and found to be O_2-limited and temperature-dependent. Excess concentration of Mn^{2+} inhibited its oxidation (Emerson et al., 1982; Tebo and Emerson, 1985). Removal of manganese from solution involved both binding to particulates (bacterial cells and inorganic aggregates) and oxidation of the manganese. The oxidation state of particulate manganese in the water samples upon collection was in the range of 2.3–2.7, suggesting the possible in situ formation of Mn(III) and mixed Mn(II)-Mn(IV) oxides (Emerson et al., 1982). Mn(II) binding and oxidation were accompanied by Co(II) binding in the chemocline (Tebo et al., 1984).

In Framvaren Fjord, Norway, evidence of bacterial Mn^{2+} oxidation has also been observed above the chemocline, which is located in the euphotic zone at a depth of 17 m and persists all year (Jacobs et al., 1985). The manganese oxidation rate measured as O_2-dependent ^{54}Mn binding was greater than in Saanich Inlet (Tebo et al., 1984).

Manganese Oxidation in the Mixed Layer of the Ocean

A comparison of the manganese-oxidizing activity in the mixed layer (waters above the thermocline) of the Sargasso Sea (Atlantic Ocean) at the Bermuda Atlantic Time Series Station (BATS) and two stations in the Pacific (0°N, 140°W and 9°N, 147°W) in 1991 (Moffett, 1997) led to the following conclusions. Particulate manganese formation in the Sargasso Sea is chiefly the result of microbial oxidation throughout the year. In the Pacific, no microbial oxidation was measured above 175 m. The formation of particulate manganese (oxide) was inhibited in the presence of azide, indicating biological involvement. The particulates were not dissolved by ascorbate. Whereas in the Pacific, light stimulated nonoxidative biological $^{54}Mn^{2+}$ uptake suggesting phytoplankton involvement, in the Sargasso Sea light inhibited oxidative biological uptake. Interestingly, Moffett and Ho (1996) found Mn^{2+}-oxidizing bacteria in Waquoit Bay, Massachusetts, that also have a capacity to oxidize Co^{2+} to Co^{3+}. A common enzyme appears to be involved. In the Sargasso Sea, by contrast, they found that Co was taken up by particulates nonoxidatively in a light-dependent process, suggesting phytoplankton involvement. In this body of water, Co uptake is thus completely decoupled from Mn uptake into particulates. Tebo and Lee (1993) previously reported Co^{2+} oxidation by spores of marine *Bacillus* SG-1.

Manganese Oxidation on the Ocean Floor

Manganese oxides can be found in large quantities in concretions (nodules) or crusts on the ocean floor at great distances from hydrothermal discharges, where the rate of sedimentation is low (Fig. 16.14) (Margolis and Burns, 1976). They have been found in all the oceans of the world (Horn et al., 1972). They may cover vast areas of the ocean floor, as on some parts of the Pacific Ocean floor, or be distributed in patches. Typical composition of such nodules is given in Table 16.7. The chemical components of a nodule are not evenly distributed throughout its mass (Sorem and Foster, 1972). When examined in cross section, nodules are seen to have developed around a nucleus, which may be a foraminiferal test, a piece of pumice, a shark's tooth, a piece of coral, an ear bone from a whale, an older nodule fragment, etc.

The oxidation state of manganese in deep sea nodules is mainly +4 (Murray et al., 1984; Piper et al., 1984; Pattan and Mudholkar, 1990). Manganese occurs as todorokite, birnessite, and ∂-MnO_2 (disordered birnessite, according to

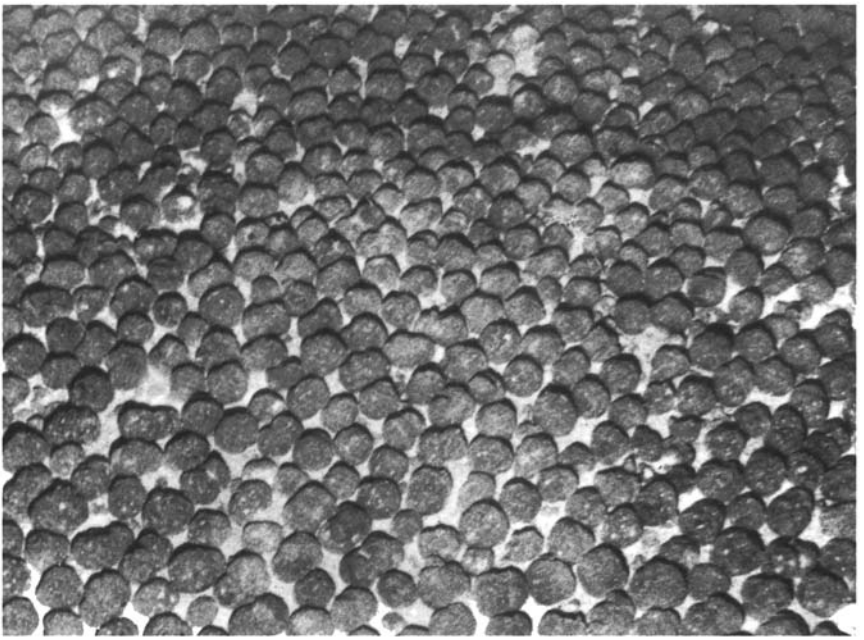


FIG. 16.14 A bed of ferromanganese nodules on the ocean floor at a depth of 5292 m in the southwest Pacific Ocean at 43°01′S and 139°37′W. Nodules may range in size from <1 to 25 cm in diameter. Average size has been given as 3 cm. (From *The Face of the Deep* by BC Heezen and C Hollister, published by Oxford University Press in 1977.)

TABLE 16.7 Average Concentration of Some Major Constituents of Manganese Nodules from the Pacific Ocean (Percent by Weight)

Mn	24.2	Mg	2.7
Fe	14.0	Na	2.6
Co	0.35	Al	2.9
Cu	0.53	Si	9.4
Ni	0.99	L.O.I.[a]	25.8

[a] L.O.I. = loss on ignition.
Source: Mero (1962).

the nomenclature of Burns et al., 1974). The Mn(IV) oxides in the nodules have a strong capacity to scavenge cations (e.g., Crerar and Barnes, 1974; Ehrlich et al., 1973; Loganathan and Burau, 1973; Varentsov and Pronina, 1973), particularly Mn^{2+} and other ions of transition metals of the first series. The nodules thus serve as concentrators of divalent manganese and some other ions in seawater and are therefore able to furnish Mn(II) in sufficient concentration to serve as an energy source to Mn(II)-oxidizing bacteria that live on nodules. This makes ferromanganese nodules a selective habitat for these bacteria and other organisms that may depend on them directly or indirectly. Electron microscopic examination and culture tests have shown the presence of various kinds of bacteria on the surface and within nodules (Fig. 16.15) (LaRock and Ehrlich, 1975; Burnett and Nealson, 1981; Ehrlich et al., 1972). Their numbers have been found to range from hundreds to tens of thousands per gram of nodule, as determined by plate counts on seawater–nutrient agar at 14–18°C and atmospheric pressure (Ehrlich et al., 1972; see also Sorokin, 1972). These numbers are probably underestimates, because the organisms grow in clumps or microcolonies on or in a nodule, from which they cannot be easily dislodged. Indeed, it is necessary to plate suspended, crushed nodule material rather than the washings of nodule fragments to make the counts.

The microbial population on nodules includes three types of bacteria when considered in terms of their action on manganese compounds (Ehrlich et al., 1972; Ehrlich, 2000). The three types are Mn(II) oxidizers (Group II, see Sec. 16.5), Mn(IV) reducers (Sec. 16.6), and a group that can neither oxidize Mn(II) nor reduce Mn(IV). In the nodules examined by Ehrlich et al. (1972), the Mn(IV) reducers were most numerous. These findings must not, however, be interpreted to mean that the Mn(IV) in the nodules examined was necessarily undergoing active reduction. Neither the Mn(II) oxidizers nor the Mn(IV) reducers are dependent on respective forms of manganese in order to grow. Thus, it is quite conceivable that the nodules were undergoing net manganese accretion at the time

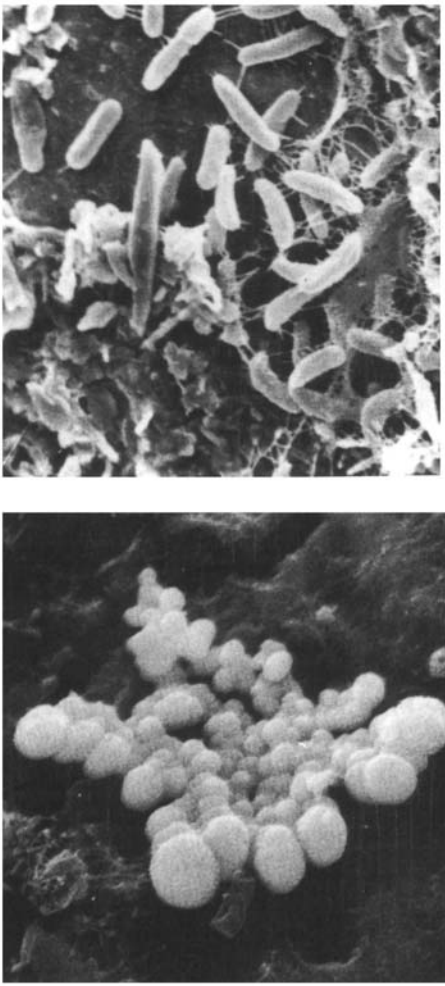


FIG. 16.15 Scanning electron potomicrographs of bacteria attached to the surface of ferromanganese nodules from Blake Plateau, off the southern Atlantic coast of the United States. Note the slime strands anchoring the rod-shaped bacteria to the nodule surface. (Reproduced from LaRock and Ehrlich, 1975. Copyright 1975 Springer-Verlag, New York, with permission.)

of collection. A plentiful supply of Mn(II) and oxygen as well as a deficit in organic carbon are needed to favor Mn(II) oxidation, whereas an adequate supply of oxidizable organic carbon is needed to favor Mn(IV) reduction. The bacteria that neither oxidize nor reduce manganese may play an important role in keeping

the level of oxidizable organic carbon low, thereby favoring the Mn(II)-oxidizing activity by the Mn(II)-oxidizing bacteria. The inability of Sorokin (1972) to culture Mn(II)-oxidizing bacteria from his nodule specimens may have had to do with the culturing method and the method of detection of manganese oxidation he employed.

Most of the organisms found on nodules are gram-negative rods (Ehrlich et al., 1972), although gram-positive bacilli, micrococci, and *Arthrobacter* have also been isolated from them (Ehrlich, 1963). A curious and as yet unexplained finding has been that a significantly greater number of isolates recovered from the central Pacific than of those from the eastern Pacific were unable to grow in freshwater media.

All Mn(II)-oxidizing bacterial cultures isolated from nodules and associated sediments by Ehrlich and associates are of the Group II type (see Sec. 16.5). They oxidize Mn^{2+} only if it is first bound to Mn(IV) oxide, ferromanganese, or certain ferric iron–coated sediment particles (Ehrlich, 1978b, 1982, 1984). As previously mentioned, Mn(IV) oxides at neutral to alkaline pH act as scavengers of cations, including Mn^{2+} ions. Given that Mn(IV) oxide is the product of manganese oxidation, the oxidation process generates new scavenging sites, and in this way it keeps nodules growing. The scavenging action of Mn(IV) oxides probably explains how other cationic constituents get incorporated into nodules:

$$H_2MnO_3 + X^{2+} \rightarrow XMnO_3 + 2H^+ \tag{16.16}$$

where X^{2+} represents a divalent cation (e.g., Cu^{2+}, Co^{2+}, Ni^{2+}) (Goldberg, 1954; Ehrlich et al., 1973; Loganathan and Burau, 1973; Varentsov and Pronina, 1973). Iron, if incorporated in this fashion, is probably picked up as Fe(III).

The initial Mn(IV) oxide formed by these bacteria is probably amorphous. The characteristic mineral assemblages identified in nodules by mineralogists are probably formed subsequently by a slow "aging" process (crystallization?) involving structural rearrangements of the nodule components (diagenesis).

The growth rate of manganese nodules in the deep sea is reportedly very slow. Ku and Broecker (1969) and Kadko and Burckle (1980), for instance, reported rates ranging from 1 to 10 mm per 10^6 years, based on radioisotope dating methods. Reyss et al. (1982) found a nodule in the Peru Basin whose rate of growth they estimated radiometrically to have been 168 ± 24 mm per 10^6 years. These estimates assume a constant rate of growth. Heye and Beiersdorf (1973) found variable growth rates for deep sea nodules that they examined by a fission track method. They reported rates varying from 0 to 15.1 mm per 10^6 years. In other words, the nodules these investigators examined did not grow at constant rates. This suggests that conditions must not be continually favorable for nodule growth and that quiescent periods, and perhaps even periods of nodule degradation, very likely aided by the Mn(IV)-reducing bacteria, must intervene

between growth periods. Bender et al. (1970) estimated manganese accumulation rates from five nodule specimens as ranging from 0.2 to 1.0 mg cm^{-2} per 1000 yr. If manganese incorporation and therefore nodule growth is an intermittent process, actual growth rates may be somewhat faster than the above estimates from radiodating, but probably by no more than an order of magnitude. Rates of manganese oxidation with bacteria isolated from nodules determined under optimal conditions in the laboratory (15°C, hydrostatic pressure of ~1 atm) were many orders of magnitude faster than any estimated in situ rates of manganese incorporation into nodules (and therefore nodule growth rates). By contrast, growth rates of nodule bacteria and bacterial manganese oxidation rates are greatly slowed in laboratory experiments by applying in situ hydrostatic pressure (e.g., 300–500 atm) and low temperature (e.g., 4°C) (Ehrlich, 1972). Hence the in situ slow growth rates of nodules are not a sufficient argument against microbial participation in nodule genesis. Conditions appear not to be continually favorable for nodule growth.

Other biogenic mechanisms of nodule growth have been evoked in the past. Butkevich (1928) tried to explain the growth in terms of iron precipitation by *Gallionella* and *Persius marinus*, the latter newly isolated by him but not otherwise known, which he found associated with brown deposits of the Petchora and White Seas. However, Sorokin (1971) concluded that *Gallionella* does not occur in deep sea nodules, nor could he find evidence for the presence of *Metallogenium*. The only types of bacteria that he detected in nodules were some other heterotrophs. The Petchora and White seas are, of course, marginal seas and untypical of the open ocean, which may explain Butkevich's findings. Novozhilova and Berezina (1984) did report finding *Gallionella* in samples from the Atlantis Deep I in the Red Sea. They found *Metallogenium*, *Caulococcus*, *Siderococcus*, and *Naumanniella* besides some other types of bacteria in samples from the northwestern Indian Ocean. Thus, these bacteria can be isolated from marine settings. It is, however, very likely that the stations from which they collected their samples were subject to continental influences that contributed these bacteria.

Graham (1959) proposed that manganese nodules form as a result of bacterial destruction of organic complexes of Mn(II) in seawater, liberating Mn^{2+} ions, which then autoxidize and precipitate, the oxides collecting around foci such as foraminiferal tests. According to Graham, other trace metals could be deposited in a similar manner. He felt that amino acids or peptides were the complexing agents that were attacked by the bacteria. He detected amino acid–like material in nodules from Blake Plateau. Graham and Cooper (1959) were able to recover foraminiferal tests from Blake Plateau, among which arenaceous ones were coated with a veneer of manganese-rich material containing Cu, Ni, Co, and Fe. Because the manganese-rich coating was on the surface of the tests, the authors reasoned that the manganese was deposited on the foraminifera after

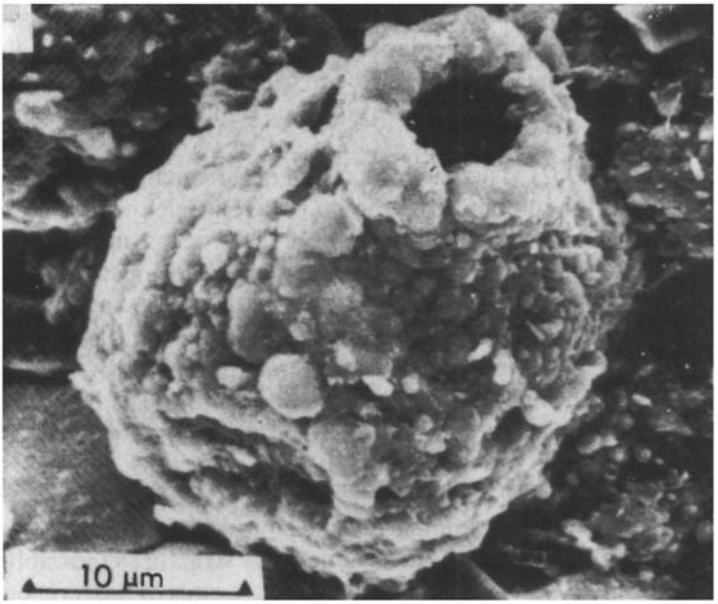
their death. They found calcareous foraminiferal tests to be free of this deposit. They implicated a protein-rich coating on the arenaceous tests as responsible for providing a habitat for organisms (bacteria?) that remove trace element chelates from seawater.

Kalinenko et al. (1962) visualized the origin of ferromanganese nodules in terms of bacterial colonies growing on ooze particles. The bacteria on these ooze particles were thought to mineralize the organic matter coating the particles. It is supposed that in the process, manganese and iron oxides are formed and deposited together with other trace elements on the colonies. Slime formed by the bacteria is assumed to cause the deposits to agglutinate, producing micronodules. The investigators studied this process in laboratory experiments by observing bacterial development on glass slides introduced into oozes from the bottom of several Indian Ocean stations. They described the bacteria as the living cement that holds the nodules together.

Ghiorse (1980) examined the surface layers of ferromanganese concretions from the Baltic Sea by scanning and transmission electron microscopy and by light microscopy. He noted filamentous structures (possibly fungal hyphae) and single cells, microcolonies, or aggregates of bacteria on the outer surface and some rods and cocci within a matrix of polymerlike material and occasionally sheathed bacteria. Budding and star-shaped bacteria and bacteria with internal membranes were also seen (possibly cyanobacteria, purple photosynthetic bacteria, or methanotrophic bacteria). The bacterial assemblage described by Ghiorse reflects a not unexpected terrestrial influence on the development in the Baltic Sea concretions and suggests an involvement of some different microbes in the growth of these nodules.

Burnett and Nealson (1983) examined the surface of some fragments from North Pacific Ocean nodules by energy-dispersive X-ray analysis. They found regions of high manganese and iron concentrations that outlined microorganism-like objects on the nodule surface. They inferred from this observation that the microorganisms were involved in accretion or removal of the metals because they would have expected the Mn and Fe to have been evenly distributed over the surface if a purely physicochemical process had been involved.

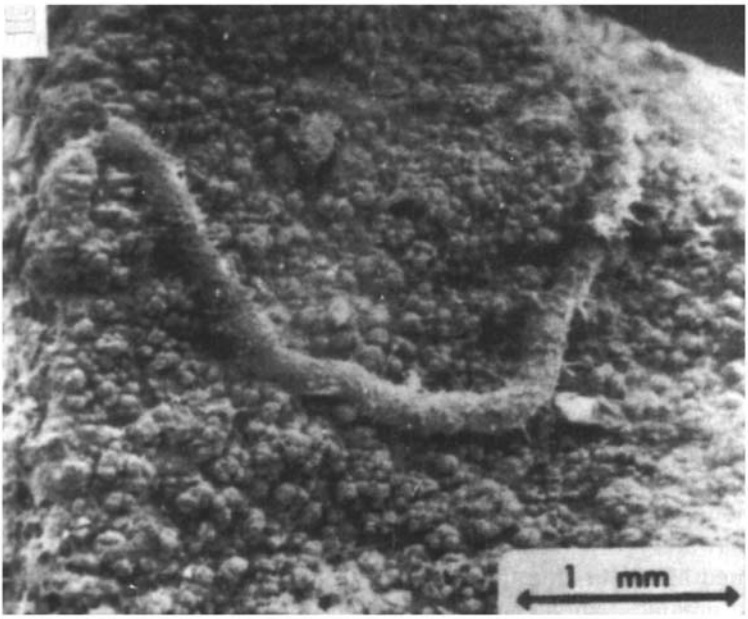
Greenslate (1974a) observed manganese deposition in microcavities of planktonic debris, especially diatoms, and proposed that such deposition was the beginning of nodule growth. He also found remains of shelter-building organisms such as benthic foraminifera on nodules, which became encrusted and ultimately buried in the nodule structure. He proposed that the skeletal remains provided a framework on which manganese and other nodule components were deposited, perhaps with the help of bacterial action (Greenslate, 1974b). Others have since reported evidence of traces of such organisms on nodules (Fredericks-Jantzen et al., 1975; Bignot and Dangeard, 1976; Dugolinsky et al., 1977; Harada, 1978; Riemann, 1983) (Fig. 16.16).



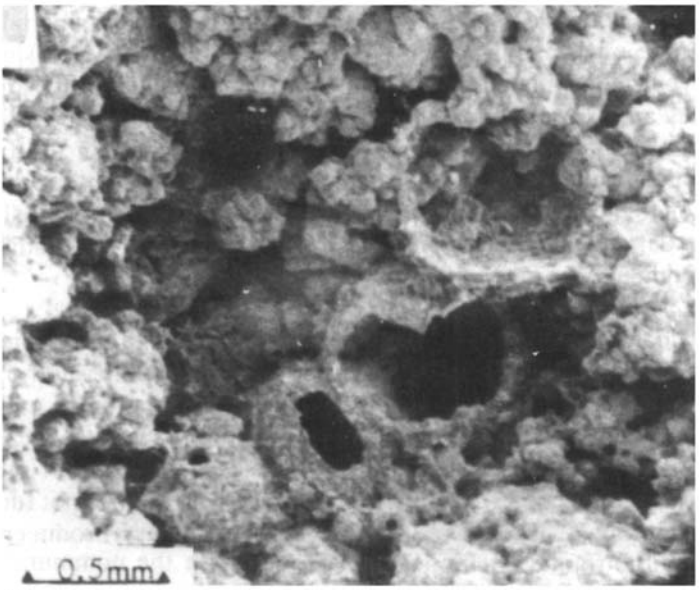
(A)

FIG. 16.16 Benthic test-forming protozoans inhabiting the surface of ferromanganese nodules. (A) Fresh remains of chambered encrusting protozoans on a nodule surface, showing siliceous biogenic material used in test construction. (B) Surface of a nodule with a partial test of *Saccorhiza ramosa*, the most common and longest of any tubular agglutinating foraminifera yet found on nodules. (C) Test of unidentified form composed almost entirely of manganese micronodules. (From Dugolinsky et al., J Sediment Petrol 47:428–445, 1977; with permission.)

The finding that benthic foraminifera and other protozoa have grown and may presently be growing on ferromanganese nodules is of significance in explaining the role of Mn(II)-oxidizing bacteria in nodule growth. Because these foraminifera and other protozoa are **phagotrophic** (i.e, they prey on living bacterial cells), they probably feed on the Mn(II)-oxidizing bacteria, among other types on the nodules. To maintain this food supply, uneaten Mn(II)-oxidizing bacteria must therefore continue to multiply. Thus, Mn(II)-oxidizing bacteria on nodules may play a dual role: (1) to aid in manganese accretion to nodules and (2) to serve as food for phagotrophic protozoa. A somewhat different interpretation of the interrelationship of bacteria, protozoa, and tube-building polychaete worms resident on nodules was offered by Riemann (1983). He found that rhizopods on the nodules accumulated large volumes of fecal pellets (stercomata) containing biogenic and mineral particles (manganese



(B)



(C)

FIG. 16.16 (*continued*)

oxides, etc.) in their tests as a result of feeding on bacteria and detritus and organic primary film on the nodule surface. He proposed that manganese oxides ingested by the protozoa are at least partially reduced, released, and reprecipitated, in part by bacterial activity on the nodule surface. He viewed polychaete worms feeding on the rhizopods as further aiding in the concentration of the manganese oxide–containing stercomata.

Although the hypotheses of Graham, Greenslate, and to some extent Kalinenko et al. may well have some bearing on the initiation of formation of some nodules, it seems doubtful that they apply to the major growth phase of nodules. Graham and Kalinenko et al. assumed that most of the Mn^{2+} in seawater is organically complexed. According to them, it is incorporated into nodules as preformed Mn(IV) oxide that resulted from autoxidation of the Mn^{2+} freed from organic complexes through ligand degradation by microbes. Incorporation of Mn(IV) oxides into nodules is also a part of the assumption of Riemann (1983), but his hypothesis does not absolutely depend on it. Sorokin (1972) also assumed that the role of heterotrophic bacteria on nodules in nodule genesis was one of digesting organic manganese complexes, releasing Mn^{2+}. However, he postulated that the Mn^{2+} becomes bound to the surface of nodules and is then abiotically oxidized. Filter-feeding benthic invertebrates that are resident on the nodules were viewed by Sorokin as the source of the organically complexed manganese. None of the proposals give recognition to the fact that most manganese in seawater, as previously pointed out, exists as inorganic complexes from which Mn^{2+} is readily adsorbed by nodules.

Manganese Oxidation Around Hydrothermal Vents

A special type of biological community driven by geothermal rather than radiant energy from the sun has been discovered around hydrothermal vents situated on mid-ocean spreading centers. Because sunlight does not reach the ocean depths where these communities exist, and because introduction of nutrients by thermal convection from overlying surface waters does not furnish sufficient nutrients, the ecosystem depends on primary producers consisting of chemolithotrophic bacteria. The consumers in the community depend on these lithotrophs, directly at the first trophic level and indirectly at other trophic levels. Indeed, at the first trophic level, many of the consumers have established an intimate symbiotic relationship with some of the primary producers (see Chap. 19 and discussions by Jannasch and Wirsen, 1979; Jannasch, 1984; and Jannasch and Mottl, 1985). The available energy sources for the primary producers (chemolithotrophic bacteria) vary, depending on the output of the vents. Potential energy sources include reduced forms of sulfur (H_2S, S^0, $S_2O_3^{2-}$), H_2, NH_4^+, NO_2^-, Mn^{2+}, CH_4, and CO (Jannasch and Mottl, 1985). Although ferrous iron is frequently a major constituent of hydrothermal fluid from black smokers (see below), it is least likely

to serve as an energy source because of its great tendency to rapidly precipitate as iron sulfide at the mouth of a vent when the hydrothermal solution meets cold, oxygenated bottom water. Most of any iron that escapes sulfide precipitation quickly autoxidizes. Available electron acceptors for bacteria in the vent community include O_2, NO_3^-, S^0, SO_4^{2-}, CO_2, and Fe^{3+} (Jannasch and Mottl, 1985) and very likely Mn(IV) oxide.

The hydrothermal solution results from deep penetration (as much as 1–3 km) of seawater into the basalt beneath the ocean floor at mid-ocean spreading centers. When a magma chamber underlies the region of seawater penetration, the heat diffusing from the chamber (the temperature may be in excess of 350°C) and the high hydrostatic pressure exerted at this site cause the seawater to react with the basalt. Seawater sulfate may be chemically reduced to sulfide by ferrous iron in the basalt (a rare example of chemical sulfate reduction in nature), bicarbonate may become reduced to methane, and Mg^{2+} from seawater may react with silica in the basalt to form talc and protons according to the reaction (Shanks et al., 1981)

$$Fe^{2+} + 2Mg^{2+} + 4H_2O + 4SiO_2 \rightarrow 6H^+ + FeMg_2Si_4O_{10}(OH)_2 \quad (16.17)$$

and render the altered seawater acidic. The acidity (protons) aids in leaching base metals (Mn^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, etc.) from the basalt. Acidity may also be generated in the formation of calcium compounds from basalt constituents and seawater calcium (Bischoff and Rosenbauer, 1983; Seyfried and Janecky, 1985). As a result, the altered seawater (hydrothermal solution) becomes loaded with H_2S, Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, etc. (Jannasch and Mottl, 1985).

Vents on the mid-ocean spreading centers from which the hydrothermal solution enters the ocean are differentiated between so-called white smokers and black smokers. *White smokers* emit gases and a hydrothermal solution that has a temperature between 6 and 23°C at the mouth of their vents. *Black smokers* emit gases and a hydrothermal solution that has a temperature near 350°C at the mouth of their vents. In the case of white smokers, the upward moving hydrothermal solution meets downward-moving cold seawater and mixes with it before emerging from vents. As a result of this mixing with seawater, the hydrothermal solution loses a significant portion of its metal charge through precipitation in the rock stratum in which this mixing occurs. Consequently, when this mixed solution emerges from the vent, it still contains a significant amount of H_2S and Mn^{2+} but little iron. In the case of black smokers, the upward moving hydrothermal solution does not encounter significant amounts of downward moving cold seawater and is little altered when it issues from vents. On emergence and mixing with cold seawater, voluminous amounts of black iron, copper, and zinc sulfides precipitate around the mouth of a black smoker vent and may be deposited in the form of a chimney along with anhydrite ($CaSO_4$) (Fig. 16.17). Much of the iron in the hydrothermal solution precipitates as sulfide, but

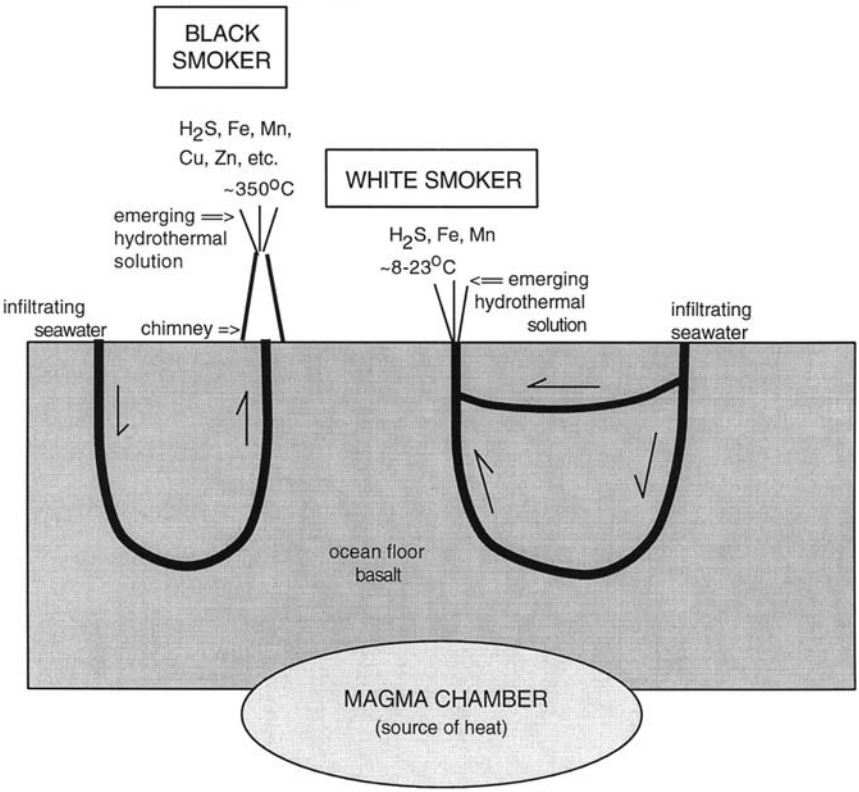


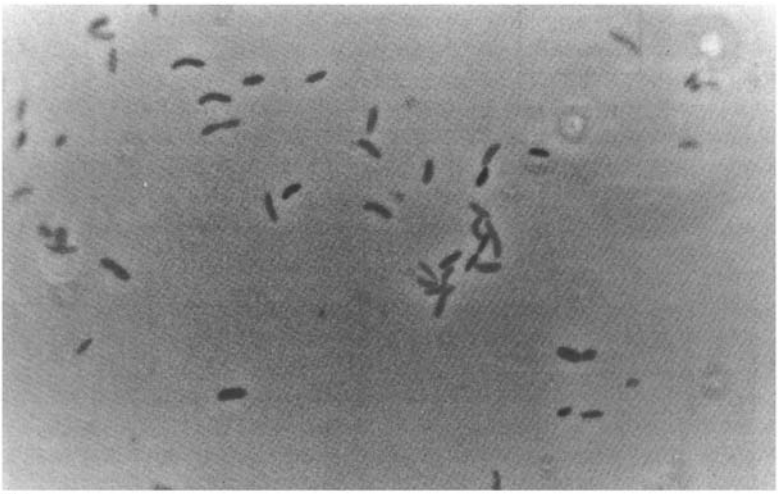
FIG. 16.17 Schematic representation of the origin of hydrothermal solutions from black smoker and white smoker vents at midocean rifts. (Adapted from Jannasch and Mottl, 1985.)

that which does not precipitates as iron oxide (FeOOH). A major portion of the manganese (Mn^{2+}) remains in solution and may be carried a considerable distance (tens of kilometers) before it is precipitated.

Although H_2S appears to be the energy source most widely used by the primary producers of vent communities at mid-ocean spreading centers, Mn^{2+}, because of its relative stability in seawater, could also be a potential energy source. Indeed, Mn^{2+}-oxidizing bacteria (Group I, Subgroup Ia, see Sec. 16.5) have been isolated from water samples collected around a white smoker (Mussel Bed Vent) on the Galapagos Rift (Ehrlich, 1983) and black smokers on the East Pacific Rise at 21° north (Ehrlich, 1985) and 10° north (Ehrlich, unpublished results). Mandernack (1992) and Mandernack and Tebo (1993) measured

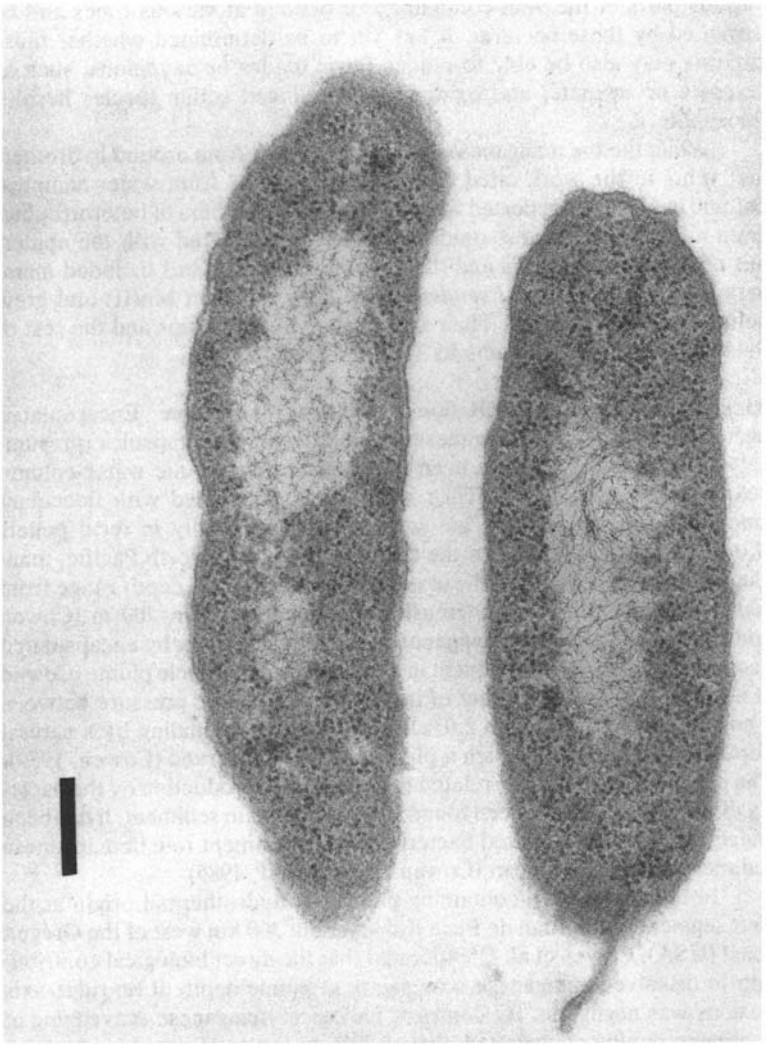
moderate to high microbial Mn removal (oxidizing activity) at the source of Mn plumes on a hydrothermal vent field on the Galapagos Rift at 50–100 m above the mouth of the vent and about 10 km distance. This study included Mussel Bed Vent. Similarly, he measured low to high microbial Mn removal (oxidizing activity) at the source of Mn plumes on the Juan de Fuca hydrothermal vent field on Endeavor Ridge at 50–200 m above the mouth of the vent and about 10 km distance.

Two bacterial isolates from around Mussel Bed Vent and three from around a vent at 21°N on the East Pacific Rise have been studied in some detail (Ehrlich, 1983, 1985). They are all gram-negative bacteria and, depending on the isolate, have the shape of short or curved rods or spirilla (Fig. 16.18). The isolates from around the white smoker grew in a temperature range of 4–37°C, and those from around the black smoker in a temperature range of 5–45°C. None grew in nutrient broth prepared in freshwater. The two isolates from Mussel Bed Vent also did not grow in nutrient broth prepared with 3% NaCl. Both isolates from Mussel Bed Vent and two of the isolates from the vent at 21°N, East Pacific Rise, are examples of Group I, Subgroup Ia (see Sect. 16.5) manganese oxidizers, and the third isolated from around the vent at 21°N, East Pacific Rise, is an example of a Group II manganese oxidizer (see Sec. 16.5). The manganese-oxidizing system



(A)

FIG. 16.18 Bacterial strain SSW_{22} isolated from a water sample from near a hydrothermal vent on the Galapagos Rift. (A) Typical morphology in a seawater medium containing tryptone, dextrose, yeast extract, and $MnSO_4$. Note range of cell size, typical of growth in this medium (×1500). (B) Longitudinal sections of SSW_{22} viewed by electron microscopy (×30,250). (Courtesy of W. C. Ghiorse.)



(B)

FIG. 16.18 Continued.

was inducible in all isolates, even in the Group II manganese oxidizer. This is in contrast to all Group II manganese oxidizers isolated from ferromanganese nodules and associated sediments in which the manganese-oxidizing system was constitutive. All five vent isolates appear to be able to obtain energy from Mn(II) oxidation. This conclusion was reached on the basis of inhibition studies

of manganese oxidation with electron transport inhibitors, and in the case of the Mussel Bed Vent isolates by direct determination of ATP synthesis coupled to Mn^{2+} oxidation (Ehrlich, 1983, 1985). In the Mussel Bed Vent isolates, ATP synthesis was very tightly coupled to Mn^{2+} oxidation, and growth in batch culture showed significant stimulation by Mn^{2+} in initial stages, which may be attributable to mixotrophy (Ehrlich and Salerno, 1988, 1990).

The two isolates from around Mussel Bed Vent were metabolically very versatile. In addition to being able to oxidize Mn^{2+}, they were also able to reduce MnO_2 aerobically and anaerobically with glucose, succinate, and acetate as electron donors (Ehrlich et al., 1991; Ehrlich, unpublished data). Their MnO_2 reductase was inducible. They were also able to oxidize thiosulfate aerobically and reduce tetrathionate anaerobically (Tuttle and Ehrlich, 1986). The ability to use this variety of inorganic electron donors and acceptors suggests that these organisms are opportunists that will use whatever energy source or electron acceptor is in plentiful supply. Mn^{2+} emitted by the vents, thiosulfate and tetrathionate formed in partial chemical or biological oxidation of H_2S emitted by the vents, or MnO_2 resulting from biological or chemical oxidation can all be expected to occur in various parts of the vent community or beyond at various times and be exploited by these bacteria. It has yet to be determined whether these bacteria may also be able to reduce ferric oxides or oxyanions, such as arsenate or selenate, and oxidize reduced sulfur species besides thiosulfate.

Whereas the manganese-oxidizing cultures from around hydrothermal vents in the work cited above were obtained from water samples, Durand et al. (1990) reported finding significant numbers of heterotrophic, gram-negative manganese-oxidizing bacteria associated with the epidermis of polychaete worms and their tubes from vent sites. The bacteria included members of *Aeromonas* and *Pseudomonas*. They oxidized Mn(II) and grew better at 40°C than at 20°C. Their significance to the worms and the rest of the vent community remains to be established.

Bacterial Manganese Precipitation in the Seawater Column

Encapsulated bacteria with iron and manganese precipitates in their capsules (presumably their glycocalyx) have been detected in the oceanic water column below 100 m (Fig. 16.19). They were found associated with flocculent amorphous aggregates (marine snow) and occasionally in fecal pellets (Cowen and Silver, 1984). In the eastern subtropical North Pacific, manganese deposits on bacterial capsules were absent in a depth range from 100 to 700 m but became increasingly noticeable below 700 m (Cowen and Bruland, 1985). Such manganese-scavenging activity by encapsulated bacteria was also very prominent in a hydrothermal vent plume (Cowen et al., 1986). A positive effect of increased hydrostatic

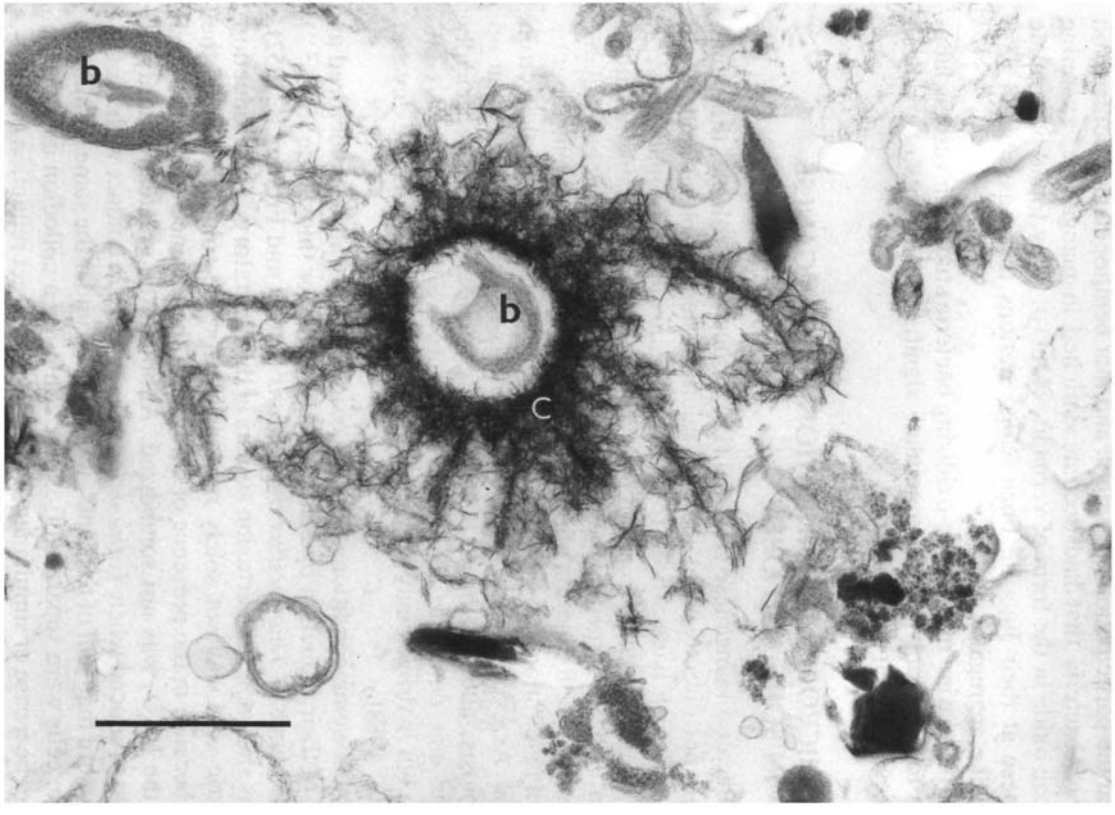


FIG. 16.19 Marine bacteria encapsulated in manganese oxide. Transmission electron photomicrograph. b, bacterial cell; c, bacteria capsule. Scale bar = 1 μm. (From Cowen et al., 1986. Copyright 1986 Macmillan Magazines Ltd.)

pressure between 1 and 200 bar (1.01×10^2 to 2.03×10^4 kPa) on Mn^{2+} binding by a natural population of bacteria in such a plume has been observed (Cowen, 1989). The pressure effect may be related to exopolymer production by the bacteria. Because bacterial capsules have been found to be abundant in sediment, it has been suggested that encapsulated bacteria play a prominent role in manganese sedimentation in the ocean (Cowen and Bruland, 1985).

In the case of Mn-containing plumes of hydrothermal origin at the cleft segment of the Juan de Fuca Ridge, about 300 km west of the Oregon coast, Cowen et al. (1990) found that the direct biological contribution to dissolved manganese scavenging at plume depth at stations on the ridge axis was negligible. By contrast, biological manganese scavenging at nonplume depths contributed almost 50% to the total process. At most off-ridge axis stations, most of the dissolved manganese scavenging was almost totally biological at plume depth. Manganese scavenging rates from a vent plume ranged from 1.7 to 3.4 mM m^{-2} yr^{-1}. In Gorda Ridge event plumes, Cowen et al. (1998) found that the ratio of encapsulated Mn-precipitating bacteria to total bacteria was greater in a days-old plume than in older plumes. The higher ratio in recently formed plumes may have been due to resuspension of encapsulated bacteria in disturbed sediment, whereas the lower ratio in older plumes was due to aggregation and particle settlement.

Tambiev and Demina (1992) found dissolved manganese to be much more rapidly converted to particulate manganese in the southern trough of the Guaymas Basin, Gulf of California (Sea of Cortez), than in the caldera of the Axial Mountain on the Juan de Fuca Ridge. They suggested that the rapid conversion of dissolved manganese in the Guaymas Basin involved participation of bacteria and that benthopelagic zooplankton grazed on bacteria that had become enriched in manganese, excreting manganese oxide in the form of vernadite (MnO_2). They found particulate manganese at their Juan de Fuca Ridge stations to be associated with bacteria-like aggregates.

Despite the foregoing report of manganese oxide deposition on bacterial envelopes, it should be stressed that of the marine manganese-oxidizing bacteria from various sources studied in various laboratories to date, only some accumulated significant amounts of oxidized manganese on their cell envelope (see discussion by Ehrlich, 1999).

16.11 MICROBIAL MOBILIZATION OF MANGANESE IN SOILS AND ORES

Soils

Because manganese(III) and (IV) oxides are water-insoluble, their manganese is not directly available as a micronutrient to plants and to soil microbes, which have

a need for it in their nutrition. The manganese in these oxides must be reduced to manganese(II) to be available nutritionally. Mn^{2+} that is sorbed by the oxides as well as by clays may become available by ion exchange. Reduction of manganese oxides may be brought about by bacterial respiration in which Mn(IV) or Mn(III) oxide replaces oxygen as terminal electron acceptor. As explained earlier, this process is not always dependent on anaerobiosis, although bacteria that reduce MnO_2 exclusively under anaerobic conditions exist and may dominate in a given environment. The electron donors are usually organic compounds, but hydrogen may serve under anaerobic conditions (Sec. 16.6) (Lovley and Goodwin, 1988).

Under some conditions, Mn(IV) oxides in soil can be solubilized as a result of their reduction by elemental sulfur (S^0) or thiosulfate ($S_2O_3^{2-}$) in the presence of *Thiobacillus thiooxidans* (Vavra and Frederick, 1952). This was shown in soil perfusion studies in the laboratory. The production of sulfuric acid was not solely responsible for the solubilization of the MnO_2, because in the presence of acid but in the absence of any reduced sulfur the MnO_2 was not solubilized. Although the greatest quantity of MnO_2 was reduced when *T. thiooxidans* cells were in contact with MnO_2, slightly more than half of the MnO_2 was reduced under the same conditions when the cells were separated from the MnO_2 by a collodion membrane. This may be a case of simultaneous direct and indirect reduction of MnO_2, the indirect reduction being the result of chemical reaction with partially reduced sulfur species generated in the bacterial oxidation of sulfur. A field study in Indiana, U.S.A., confirmed that S^0 and $S_2O_3^{2-}$ can mobilize fixed manganese in an agriculturally manganese deficient soil (Garey and Barber, 1952).

As previously pointed out, ferrous iron and H_2S produced in microbial iron(III) and sulfate respiration can chemically reduce Mn(IV) oxides in soils and sediments (Burdige and Nealson, 1986; Lovley and Phillips, 1988b; Myers and Nealson, 1988b; Nealson and Saffarini, 1994). Reduction of manganese oxides of microbial origin in soil can also be brought about chemically by some constituents of root exudates as from oats and vetch, especially at acid pH (Bromfield, 1958a, 1958b). Bacteria are apparently not needed to promote Mn(IV) reduction by active root exudate components (Bromfield, 1958a). Malate is an active root exudate component that has been shown to reduce Mn(IV) oxide chemically (Jauregui and Reisenauer, 1982). However, in view of the widespread ability of various types of bacteria to reduce MnO_2 and their widespread occurrence, bacterial reduction of oxides of Mn(IV) in soil is probably a more important factor in remobilizing fixed manganese in nature.

Enumeration of viable manganese reducers in soil is an incompletely solved problem. Attempts have been made to use heterotrophic agar medium containing hydrous manganese oxide for differential plate counts (e.g., Schweisfurth, 1968). MnO_2-reducing colonies have been identified as those that develop a clear halo around them, suggesting reduction of the manganese oxide in the area of the halo. The absence of manganese oxide in the halo can be confirmed by

applying berbelin blue or leucocrystal violet reagent to the colony (Krumbein and Altmann, 1973; Kessick et al., 1972). Although the clear halo indeed indicates manganese reduction enzymatic reduction cannot be inferred, because manganese reductase, which insofar as is known is not an extracellular enzyme, cannot work over a distance but must be in physical contact with any manganese oxide whose reduction the enzyme catalyzes. This means that a manganese-reducing cell must be in physical contact with it. Reduction that produced the halo must therefore be interpreted as due to nonenzymatic reaction. If the medium contains glucose, the manganese oxide reduction may be merely the result of reaction with residual glucose at acid pH generated by the colony, but it could also be due to reaction with one or more metabolic products formed by the bacterial colony from glucose. Potential enzymatic reduction in the same medium may be indicated if an artificial electron shuttle such as ferricyanide is added to the medium at low concentration. In that instance, a manganese oxide–free halo around a colony might indicate enzymatic or nonenzymatic reduction or both. If enumeration is done on plates with and without added ferricyanide, a not very discriminating differential count of enzymatic and nonenzymatic Mn(IV)-reducing organisms is possible (Tortoriello, 1971).

Ores

The autotroph *Thiobacillus thiooxidans*, when acting on elemental sulfur, has been shown to be able to extract manganese from ores (Imai and Tano, 1967; Ghosh and Imai, 1985a, 1985b). Imai and Tano leached an ore containing MnO_2 (10.6%), Fe_2O_3 (25%), SiO_2 (55%), MgO (5.23%), and traces of Ca, Al, and S. Ground ore was suspended at a concentration (pulp density) of 3% in a culture medium containing K_2HPO_4 (0.4%), $MgSO_4$ (0.03%), $CaCl_2$ (0.02%), $FeSO_4$ (0.001%), $(NH_4)_2SO_4$ (0.2%), and S^0 (1%). Addition of FeS or $FeSO_4$ stimulated both the growth of *T. thiooxidans* and the solubilization of manganese, whereas the addition of ZnS stimulated only the solubilization of manganese; $Fe_2(SO_4)_3$ was without effect. The reducing activity of partially reduced sulfur species like thiosulfate or sulfite produced by *T. thiooxidans* in the oxidation of elemental sulfur to sulfuric acid was probably the basis for the solubilization of the MnO_2 in the ore.

Ghosh and Imai (1985a, 1985b) leached manganese from MnO_2 (>90% pure, −400 mesh size) with *Thiobacillus ferrooxidans* as well as *T. thiooxidans* in medium containing the mineral salts mixture of 9K medium (see Chap. 15) with 1% elemental sulfur but without ferrous sulfate (Silverman and Lundgren, 1959). As with *T. thiooxidans*, leaching was probably the result of production of partially reduced, soluble sulfur species, such as SO_3^{2-} produced from S^0 by *T. ferrooxidans* strain AP19-3 (Sugio et al., 1988a, 1988b), which then reduced MnO_2 to Mn^{2+}. They were able to leach manganese from MnO_2 when S^0 in their

medium was replaced by chalcocite (Cu_2S) or covellite (CuS) ground to −100 mesh size. Increasing the *pulp density* (particle concentration) of MnO_2 from 0.5% to 5% increased the amount of manganese leached from MnO_2 but decreased the amount of Cu leached from chalcocite or covellite (Ghosh and Imai, 1985b).

Heterotrophic leaching of manganese ores was first attempted by Perkins and Novielli (1962). They isolated a *Bacillus* that was able to solubilize manganese from a variety of ores in an organic culture medium that contained molasses as one ingredient. The mineralogy of the ores they tested was not reported, and thus it is not clear whether reduction of manganese was involved or solubilization of manganous minerals. Heterotrophic leaching that did involve reduction of manganese oxides was reported by Trimble and Ehrlich (1968), Ehrlich et al. (1973), Agate and Deshpande (1977), Mercz and Madgwick (1982), Holden and Madgwick (1983), Kozub and Madgwick (1983), Babenko et al. (1983), Silverio (1985), Rusin et al. (1991a, 1991b); and others.

16.12 MICROBIAL MOBILIZATION OF MANGANESE IN FRESHWATER ENVIRONMENTS

Detection of manganese-reducing bacteria in some of the Karelian lakes (former U.S.S.R.) led to the inference that manganese reduction is occurring in these lakes (Sokolova-Dubinina and Deryugina, 1967a; Troshanov, 1968). In Lake Punnus-Yarvi, *Bacillus circulans*, *B. polymyxa*, and an unidentified non-spore-forming rod were thought to be involved in the formation of rhodochrosite ($MnCO_3$) from bacteriogenic manganese oxide. The manganese-reducing activity that led to $MnCO_3$ formation was found to occur on the shoreward side of a depression in Punnus Ioki Bay and was related to the bacterial degradation of plant debris originating from plant life along the shore of the lake. The manganese oxides appeared to act as terminal electron acceptors. CO_2 from the mineralization of organic matter contributed the carbonate. Limited bacterial sulfate reduction was thought to assist the process by helping to maintain reducing conditions. As much as 5% $MnCO_3$ has been found in the sediment at the active site (see also Chap. 8). Reduction of MnO_2 with acetate by the action of *Geobacter metallireducens* under anaerobic conditions, leading to the production of $MnCO_3$ identified as rhodochrosite, was demonstrated in the laboratory (Lovley and Phillips, 1988a). *G. metallireducens* was isolated from Potomac River sediment. In these instances Mn was mobilized and at least a portion reimmobilized.

Microbial Mn(IV)-reducing activity has been detected in sediments of Blelham Tarn in the English Lake District. A malate-fermenting *Vibrio* was isolated that could use Mn(IV), Fe(III), and NO_3^- as terminal electron acceptors

(Jones et al., 1984). In laboratory experiments, this organism exhibited a 20% greater molar growth yield anaerobically on Mn(IV) oxide than on Fe(III). Mn(IV) oxide and NO_3^- inhibited reduction of iron(III) by the organism. Similar inhibition of iron(III) reduction was observed when a sample of lake sediment was emended with Mn_2O_3 (Jones et al., 1983).

Davison et al. (1982) reported microbial manganese reduction in the deeper sediment of Esthwaite Water (U.K.). It was intense during winter months when manganese oxides accumulated transiently in the sediment. In the summer months, manganese oxides generated in the water column were reduced in the hypolimnion before reaching the sediment. Myers and Nealson (1988a) found a *Shewanella* (formerly *Alteromonas*, *Pseudomonas*) *putrefaciens*, isolated from Lake Oneida (New York) that, although it is a facultative aerobe, reduces Mn(IV) oxide only anaerobically with a variety of organic electron donors. It is probably one of the organisms responsible for recycling oxidized manganese in the lake. Gottfreund et al. (1983) detected manganese-reducing bacteria in groundwater to which they attributed Mn(III)-reducing ability.

16.13 MICROBIAL MOBILIZATION OF MANGANESE IN MARINE ENVIRONMENTS

Manganese(IV)-reducing bacteria have been isolated from seawater, marine sediments, and deep sea ferromanganese concretions (nodules). To date, all isolates tested in the laboratory have been heterotrophs or mixotrophs that can use one or more of the following electron donors: glucose, lactate, succinate, acetate, or the inorganic donor H_2. In 1988, in situ observations suggested that anaerobically respiring thiobacilli and some other bacteria in anaerobic sediment at the edge of a salt marsh on Skidaway Island, Georgia, catalyze the reduction of Mn(IV) oxides that are in contact with sulfide in a *solid phase* (e.g., FeS). The Mn(IV) oxide was thought to act as electron acceptor when the sulfide was oxidized to sulfate. The process was inferred from experiments in which microbial reduction of Mn(IV) oxide by sulfide was inhibited by azide and 2,4-dinitrophenol (Aller and Rude, 1988). A process resembling this has since been demonstrated in the laboratory with elemental sulfur as electron donor (Lovley and Phillips, 1994a). Organisms capable of catalyzing this reaction include *Desulfovibrio desulfuricans*, *Desulfomicrobium baculatum*, *Desulfobacterium autotrophicum*, *Desulfuromonas acetoxidans*, and *Geobacter metallireducens*. Stoichiometric transformation according to the reaction

$$S^0 + 3MnO_2 + 4H^+ \rightarrow SO_4^{2-} + 3Mn^{2+} + 2H_2O \qquad (16.18)$$

was demonstrated with *D. desulfuricans*. Fe(III) could not replace MnO_2 as electron acceptor in this reaction. This activity is not only of significance for the

marine manganese cycle but also presents an important mechanism by which sulfate can be regenerated from reduced forms of sulfur anaerobically in the dark.

Hydrogen sulfide (H_2S) produced anaerobically by sulfate-reducing bacteria has been shown to be able to reduce Mn(IV) oxide nonenzymatically, with S^0 being the chief product of sulfide oxidation (Burdige and Nealson, 1986). In addition, H_2S produced by bacterial disproportionation of elemental sulfur into H_2S and SO_4^{2-} has been shown to reduce Mn(IV) oxide chemically. Indeed, this reaction with Mn(IV) oxide [or Fe(III)] appears to be thermodynamically essential in promoting continued bacterial disproportionation of the elemental sulfur (Thamdrup et al., 1993).

Most manganese(IV)-reducing bacterial isolates from marine environments studied to date have been aerobes that can reduce MnO_2 aerobically or anaerobically, but some evidence for strictly anaerobic reduction has been obtained (see reviews by Ehrlich, 1987; Burdige, 1993). Caccavo et al. (1992) reported the isolation of a facultative anaerobe, strain BrY (now *Shewanella alga* BrY), from the Great Bay Estuary, New Hampshire, that reduced Mn(IV) oxide only anaerobically with hydrogen or lactate. The extent of in situ activity of marine MnO_2-reducing bacteria has so far not been estimated in any part of the marine environment.

Because Mn(IV) oxides are good scavengers of trace metals such as Cu, Co, and Ni, it is noteworthy that bacterial reduction of the manganese oxide in ground ferromanganese nodules in laboratory experiments resulted in solubilization of these metals along with manganese (Fig. 16.20) (Ehrlich et al., 1973). Ni and Co solubilization was absolutely dependent on Mn(IV) reduction. Cu release, on the other hand, was only partially dependent on it, being initially solubilized by complexation with peptone in the culture medium. Only in later stages did Cu solubilization show a direct dependence on bacterial action. This finding suggests that Cu may be more loosely bound in the nodule structure than Ni or Co. The need for bacterial action in Cu release appears to arise only when it is encapsulated by Mn(IV) oxides and not in direct contact with the solvent. Also noteworthy in these experiments was the observation that only negligible amounts of iron were solubilized, even though the Mn(IV)-reducing organisms used in these studies also had the capacity to reduce limonite and goethite. Whether this apparent inability to solubilize iron was due to an inability of the bacteria to reduce it in the nodules or to chemical reoxidation of any Fe(II) produced from the nodules by remaining Mn(IV) oxide in the nodules or to immediate autoxidation and precipitation after formation from the nodules is not known. However, on the basis of standard free energy yield at pH 7.0 ($\Delta G^{0'}$) from the reduction of iron oxide minerals, the first explanation is the most plausible. The solubilization of the scavenged trace metals by bacterial ferromanganese reduction may be of nutritional importance in the ecology of the marine environment where manganese oxides such as ferromanganese occur.

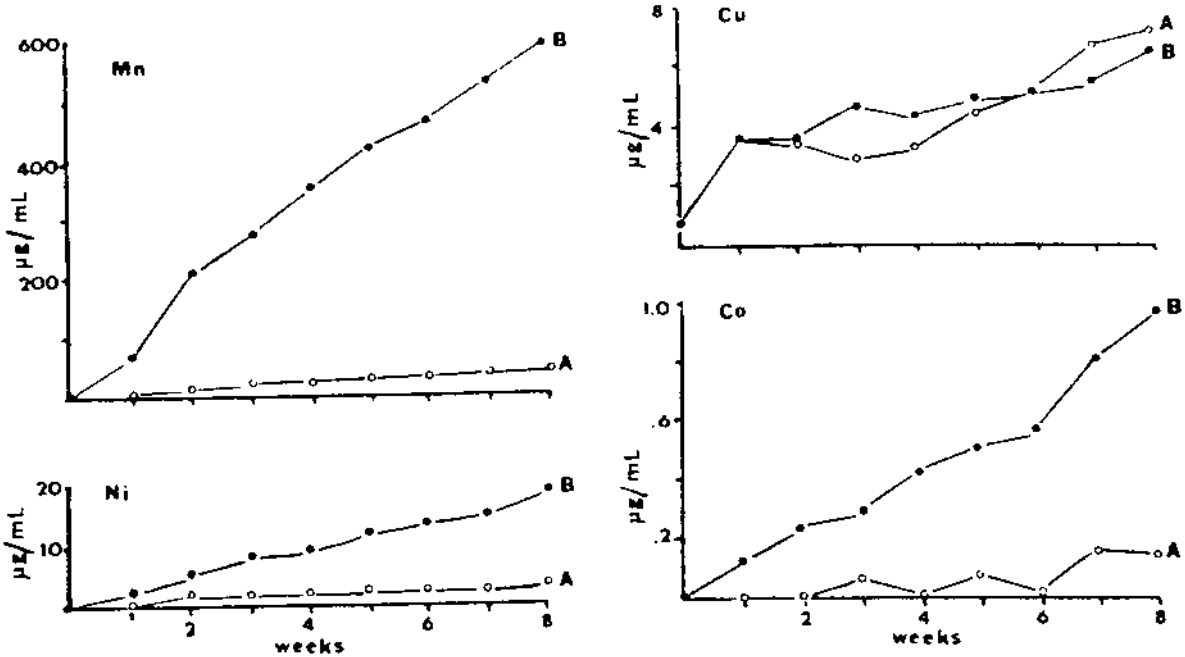


FIG. 16.20 Manganese, copper, nickel, and cobalt release from ferromanganese nodule substance by *Bacillus* GJ33 in seawater containing 1% glucose and 0.05% peptone. A, uninoculated; B, inoculated. (From Ehrlich HL, Yang SY, Mainwaring JD Jr. Bacteriology of manganese nodules, VI. Fate of copper, nickel, cobalt and iron during bacterical and chemical reduction of the manganese(IV). Z Allg Mikrobiologie 13:39–48. Copyright 1973, with permission.)

16.14 MICROBIAL MANGANESE REDUCTION AND MINERALIZATION OF ORGANIC MATTER

As discussed earlier (Sec. 16.6), enzymatic manganese reduction by bacteria is often a form of respiration that can occur aerobically or anaerobically, depending on the type of organism and on prevailing environmental conditions. Organic electron donors in this bacterial process can be any of a variety of different compounds. Even anaerobically, they may be completely degraded to CO_2 by a single species of organism (Lovley et al., 1993b), but often complete degradation requires the successive action of two or more species (see, e.g., Lovley et al., 1989). Thus manganese(IV) oxide respiration can be viewed environmentally as a form of mineralization of organic compounds (Ehrlich, 1993a, 1993b; Nealson and Saffarini, 1994). This form of mineralization is unlike that by bacterial sulfate respiration and most forms of iron and nitrate respiration because it can occur readily both aerobically and anaerobically if the appropriate organisms are present. However, it is probably only anaerobically that manganese respiration has significant impact on the carbon cycle, and then only if no other competing forms of anaerobic respiration occur (Lovley and Phillips, 1988b; Ehrlich, 1987). Thamdrup et al. (2000) found that in Black Sea shelf sediments, dissimilatory Mn reduction was the most important means of organic carbon mineralization in the surface layer down to ~1 cm depth, whereas dissimilatory sulfate reduction was the exclusive carbon mineralization process below this depth. The Mn respiration was accompanied by $MnCO_3$ formation.

16.15 MICROBIAL ROLE IN THE MANGANESE CYCLE IN NATURE

It must be inferred from the widespread occurrence of manganese-oxidizing and -reducing microorganisms in terrestrial, freshwater, and marine environments that they play an important role in the geochemical cycle of manganese. At neutral pH under aerobic conditions, manganese-oxidizing microbes clearly are more important in immobilizing manganese in soils and sediments than are iron-oxidizing microbes in immobilizing iron in view of the relative resistance of Mn(II) to autoxidation at pH values below 8 in contrast to Fe(II) (see, e.g., Diem and Stumm, 1984) (see also Chap. 15). The manganese-reducing bacteria are important at neutral pH because manganese oxides under reducing conditions in the absence of strong reducing agents such as H_2S or Fe^{2+} are relatively stable. As Figure 16.21 shows, manganese oxidation reactions generally lead to manganese fixation because most Mn(III) and all Mn(IV) products are insoluble. Gottfreund and Schweisfurth (1983) did suggest that soluble Mn(III) complexes may be formed in microbial manganese oxidation. By contrast, reduction of Mn(III) and Mn(IV) oxides generally leads to solubilization of manganese. Under

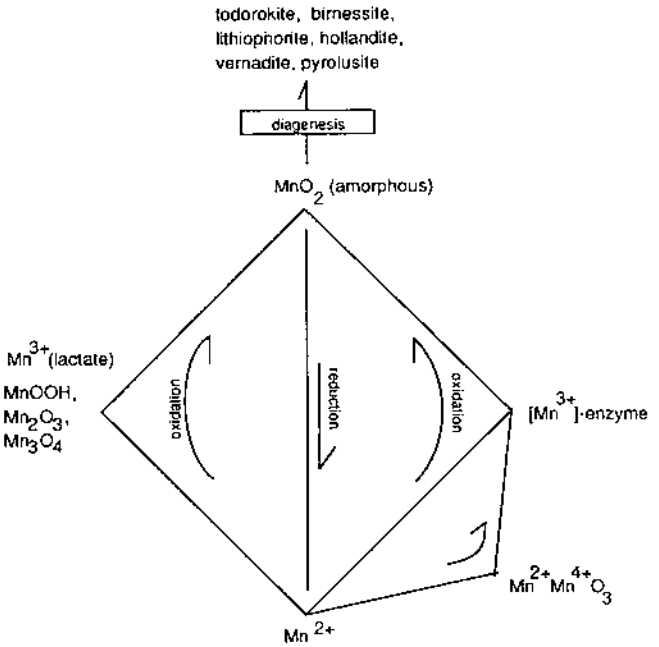


FIG. 16.21 The manganese cycle. The oxidation reactions on the left side of the diagram involve a dismutation (disproportionation) reaction of Mn(III), whereas the oxidation reactions on the right side of the diagram involve direct oxidation of enzyme-bound Mn(III). Mn(IV) reduction may or may not involve Mn(III) as an intermediate. Mn(III) oxide reduction by bacteria appears to occur but is not indicated in this diagram.

some reducing conditions, the solubilized manganese may, however, precipitate as $MnCO_3$ (see Sokolova-Dubinina and Deryugina, 1967a; Lovley and Phillips, 1988a) (see also Sec. 16.9).

Studies of biogeochemical aspects of the manganese cycle in soil and aquatic environments are still few. One reason for this is a difficulty in methodology. Two approaches are being taken. One is the reconstruction of the process in laboratory experiments. Thus, manganese and iron reduction and oxidation cycles in an acidic (pH 5.6) flooded coastal prairie soil of southwest Louisiana were reproduced in the laboratory under controlled pH and E_h conditions (Patrick and Henderson, 1981). The results showed that the rate of redox potential change could affect the oxidation and reduction rates differentially if the change was too rapid. Some Mn(IV) reduction in soils may be caused nonmicrobiologically by interaction with nitrite of microbial origin in which Mn(IV) may be reduced to Mn(II) and/or Mn(III), depending on the nitrite

concentration relative to Mn(IV) oxide (Bartlett, 1981). At high MnO_2/nitrite ratios, Mn(III) has been found to predominate.

$$NO_2^- + MnO_2 + 2H^+ \rightarrow Mn^{2+} + NO_3^- + H_2O \quad (16.19a)$$

$$NO_2^- + 2MnO_2 \rightarrow NO_3^- + Mn_2O_3 \quad (16.19b)$$

The complete reduction to Mn^{2+} is favored by acidic conditions, whereas the reduction to Mn_2O_3 is independent of pH. Microcosm experiments with surface sediment from Long Island Sound, led Hulth et al. (1999) to speculate on biochemical coupling of anaerobic lithotrophic nitrification ($NH_4^+ \rightarrow NO_3^-$) to MnO_2 reduction ($MnO_2 \rightarrow Mn^{2+}$).

Luther et al. (1997) studied denitrification (dinitrogen formation) in marine sediments resulting from interaction of MnO_2 with NH_3 and organic N, and Mn^{2+} with NO_3^-. These reactions are not sensitive to the presence of O_2 in air. According to Luther et al. (1997), denitrification due to oxidation of NH_3 and organic nitrogen by MnO_2 can predominate over nitrification ($NH_3 \rightarrow NO_3^-$) in continental margin sediments and may account for 90% of the nitrogen formed in this environment. What role, if any, microbes play in these denitrifications remains to be elucidated. Observations by Vandenabeele et al. (1995) on stimulation of manganese removal from sand filters in water purification plants by nitrate suggest that bacteria can play a role.

The second approach to studying biogeochemical aspects of the manganese cycle are in situ investigations (Nealson et al., 1988). Some examples were mentioned earlier in this chapter (Lake Punnus-Yarvi; Lake Oneida; Saanich Inlet; Framvaren Fjord). In the studies of Lake Oneida, Saanich Inlet, and Framvaren Fjord, microbial Mn(II) oxidation was demonstrated by manganese removal in the presence and absence of cell poisons from water samples collected at various depths and spiked with $^{54}Mn^{2+}$. The metabolic poisons included sodium azide, penicillin G, and tetracycline HCl. A critical evaluation of poisons of potential use as inhibitors of manganese oxidation in environmental samples was made by Rosson et al. (1984). The removal of manganese reflected both binding of manganese(II) to particulates (bacterial cells and organic and inorganic aggregates) and oxidation of Mn(II). Binding was distinguished partially from oxidation by use of formaldehyde. The oxidation state of particulate manganese in the water samples upon collection was in the range of 2.3–2.7, suggesting possible in situ formation of Mn(III) and mixed Mn(II)-Mn(IV) oxides (Emerson et al., 1982). An even better assessment of manganese oxidation rate can be obtained by measuring Mn(II) removal from solution in the presence and absence of oxygen instead of using metabolic poisons (Tebo and Emerson, 1985). In the absence of oxygen, Mn(II) removal should be due only to surface binding. Thus the difference between total Mn(II) removal in air and Mn(II) removal in the absence of oxygen should represent the amount of manganese that was oxidized.

An important question in the biogeochemical cycle of manganese is the oxidation state of the manganese in the first detectable product of biological and chemical oxidation of Mn(II). Diem and Stumm (1984) and Tipping et al. (1984, 1985) found that Mn(IV) oxide was formed microbiologically as the first recognizable oxidation product in manganese-containing water samples. Tipping et al. (1984, 1985) found that the oxide they recovered resembled vernadite (MnO_2). It had an oxidation state of 3.5. By contrast, Hastings and Emerson (1986) concluded from experiments with spores of marine *Bacillus* SG-1 that Mn^{2+} was transformed to an amorphous Mn(III) oxide that recrystallized to hausmannite (Mn_3O_4 or $MnO_{1.33}$), especially in an excess of Mn(II) (Mann et al., 1988). However, subsequent work by Mandernack (1992) and Mandernack et al. (1995) suggested that the immediate product of manganese oxidation by the spores is Mn(IV) oxide. The oxidation is caused by an exosporium protein of the SG-1 spores (Tebo et al., 1988; Mann et al, 1988; Francis and Tebo, 1999). The first stages of the Mn(II) oxidation by the exosporium protein may be enzymatic, but once the spore is covered by the insoluble manganese oxide, further oxidation is abiotic (de Vrind et al., 1986b; Mann et al., 1988).

Because the first oxidation product of abiotic Mn^{2+} oxidation was found to be Mn_3O_4,

$$3Mn^{2+} + 3H_2O + 0.5O_2 \rightarrow Mn_3O_4 + 6H^+ \tag{16.20}$$

which subsequently may disproportionate at a very slow rate to MnO_2 and Mn^{2+} under appropriate conditions of pH,

$$Mn_3O_4 + 4H^+ \rightarrow MnO_2 + 2Mn^{2+} + 2H_2O \tag{16.21}$$

(Hem, 1981; Hem and Lind, 1983; Hem et al., 1982; Murray et al., 1985), the following interpretation can be offered for contrasting biological and chemical oxidation results to date. When Mn^{2+} oxidation is enzymatically catalyzed by a manganese oxidase system with energy conservation in intact cells, the reaction may be a two-step sequence in which the first step involves enzymatic oxidation of Mn^{2+} to Mn(III), with the Mn(III) existing as a bound (possibly enzyme-bound) intermediate:

$$Mn^{2+} + H^+ + 0.25O_2 \rightarrow \{Mn^3\} + 0.5H_2O$$
$$(\Delta G^{\circ\prime} = +0.61 \text{ kcal}; +2.5 \text{ kJ}) \tag{16.22}$$

This is followed by rapid enzymatic oxidation to the final, and at the same time, first detectable product, namely a form of Mn(IV) oxide, here written as MnO_2:

$$\{Mn^{3+}\} + 1.5H_2O + 0.25O_2 \rightarrow MnO_2 + 3H^+$$
$$(\Delta G^{\circ\prime} = -16.9 \text{ kcal}; -70.6 \text{ kJ}) \tag{16.23}$$

A free energy of formation value of −35 kcal is estimated for $\{Mn^{3+}\}$ (bound Mn^{3+}). In this reaction sequence, all Mn^{2+} that is oxidized is transformed to MnO_2. It is reaction (16.23) from which the energy that the cell conserves is derived (see also Ehrlich, 1999).

When Mn^{2+} is biologically oxidized by a nonenzymatic process, it is probably oxidized according to the abiotic mechanism, in which Mn(III) oxide such as Mn_3O_4 is the first detectable product of oxidation, and MnO_2, if it forms at all, is formed very slowly by disproportionation of the Mn_3O_4. The major difference between the reactions catalyzed by manganese oxidase on the one hand and by abiotic oxidation on the other is that the manganese oxidase prevents disproportionation of Mn(III) by catalyzing its complete conversion to Mn(IV) oxide at a rapid rate, whereas in the abiotic reaction Mn_3O_4 is the first detectable product, and Mn(IV) oxide, if it forms at all, is formed very slowly by abiotic disproportionation, with the result that only one-third of the reacting Mn(III) is converted to Mn(IV), the other two-thirds being reconverted to Mn(II) [reaction (16.21)]. Environmentally it is thus possible to associate rapidly formed, fresh Mn(IV) oxide deposits with biological catalysis and Mn(III) oxide deposits with abiotic reactions (Ehrlich, 1996b).

16.16 SUMMARY

Some bacteria can oxidize manganous manganese enzymatically or nonenzymatically. Enzymatically, they may oxidize dissolved Mn^{2+} with O_2, catalyzed by an inducible or a constitutive oxidase, or they may oxidize it with metabolic H_2O_2, catalyzed by catalase. They may oxidize prebound Mn^{2+} with O_2, catalyzed by a constitutive or inducible oxidase. At least some of the oxidase-catalyzed reactions yield useful energy to the bacteria. Some fungi can oxidize Mn^{2+} with an extracellular peroxidase. Oxidation of Mn^{2+} in solution occurs in soil, freshwater, and marine environments. Oxidation of prebound Mn^{2+} has so far been demonstrated only with bacteria from marine environments.

Some bacteria and fungi may promote nonenzymatic manganese oxidation by utilization of hydroxycarboxylic acids, leading to a rise in pH above neutrality followed by oxidation of Mn(II) catalyzed by residual hydroxycarboxylic acid. Others may produce other metabolic end products, which then act as oxidants of Mn(II). Still other microbes may raise the pH by deamination of organic nitrogen compounds such as amino acids to a range where the Mn(II) autoxidizes. Some cyanobacteria and algae can promote Mn^{2+} autoxidation by raising the pH of their immediate surroundings photosynthetically.

Some bacteria and fungi can precipitate preformed oxidized manganese through sorption to their cell surface or to extracellular slime.

Some bacteria can reduce Mn(IV) oxides to manganous manganese [Mn(II)] with suitable electron donors. This reduction may be aerobic and/or anaerobic depending on the bacterial strain and environmental conditions. Such reduction has been demonstrated with bacteria from soil and from freshwater and marine environments. Some bacteria and fungi can also reduce Mn(IV) oxides nonenzymatically with metabolic end products such as H_2S and Fe^{2+}, or oxalate.

Manganese-oxidizing and -reducing microorganisms play an important role in immobilization and mobilization, respectively, of manganese in soil. In some anaerobic environments, manganese-reducing bacteria may play an important role in mobilizing fixed, nutritionally unavailable manganese and in mineralization of organic matter. Fixed manganese in soil can be concentrated in concretions or in arid environments as desert varnish. Because manganese is required in plant nutrition, manganese-oxidizing and -reducing activity is ecologically very significant.

Manganese-oxidizing and -reducing bacteria can also play a significant role in the manganese cycle in freshwater and marine environments. The manganese-oxidizing microbes may contribute to the accumulation of manganese oxides on and in sediments. The oxides they form may sometimes be deposited as concretions formed around a nucleus, such as a sediment grain, a pebble, or a dead biological structure (e.g., mollusk shell, coral fragment, or other debris). Conversely, manganese-reducing microorganisms may mobilize the oxidized or fixed manganese, releasing it into the water phase. In reducing freshwater environments, in the presence of abundant plant debris the microbial reduction of manganese oxides may lead to the formation of manganous carbonate, a different form of fixed manganese.

Ferromanganese nodules on parts of the ocean floor are inhabited by bacteria. Some of these bacteria can oxidize manganese, and others can reduce it. Together with benthic foraminifera, which can be expected to feed on the bacteria and that accumulate manganese oxides on their tests and tubes, the manganese(II) oxidizers appear to constitute a biological system that contributes to nodule formation.

REFERENCES

Adams JB, Palmer F, Staley JT. 1992. Rock weathering in deserts: Mobilization and concentration of ferric iron by microorganisms. Geomicrobiol J 10:99–114.

Adams LF, Ghiorse WC. 1985. Influence of manganese on growth of a sheathless strain of *Leptothrix discophora*. Appl Environ Microbiol 49:556–562.

Adams LF, Ghiorse WC. 1986. Physiology and ultrastructure of *Leptothrix discophora* SS-1. Arch Microbiol 145:126–135.

Adams LF, Ghiorse WC. 1987. Characterization of extracellular Mn^{2+}-oxidizing activity and isolation of an Mn^{2+}-oxidizing protein from *Leptothrix discophora* SS-1. J Bacteriol 169:1279–1285.

Adams LF, Ghiorse WC. 1988. Oxidation state of Mn in the Mn oxide produced by *Leptothrix discophora* SS-1. Geochim Cosmochim Acta 52:2073–2076.

Adeny WE. 1894. On the reduction of manganese peroxide in sewage. Proc Roy Dublin Soc, Chap 27, pp 247–251.

Agate AD, Deshpande HA. 1977. Leaching of manganese ores using *Arthrobacter* species. In: Schwartz W, editor. Conference Bacterial Leaching. Gesellschaft für Biotechnologische Forschung mbH, Braunschweig-Stöckheim. Weinheim, German: Verlag Chemie, pp 243–250.

Alexandrov EA. 1972. Manganese: element and geochemistry. In: Fairbridge RW, ed. Encyclopedia of Geochemistry and Environmental Sciences. Encyclopedia of Earth Sciences Series, Vol IVA. New York: Van Nostrand Reinhold, pp 670–671.

Ali SH, Stokes JL. 1971. Stimulation of heterotrophic and autotrophic growth of *Sphaerotilus discophorus* by manganous ions. Antonie v. Leeuwenhoek 37: 519–528.

Aller RC, Rude PD. 1988. Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. Geochim Cosmochim Acta 52:751–765.

Anderson JJ, Devol AH. 1973. Deep water renewal in Saanich Inlet, an intermittently anoxic basin. Estuarine Coastal Mar Sci 1:1–10.

Arcuri EJ. 1978. Identification of the cytochrome complements of several strains of marine manganese oxidizing bacteria and their involvement in manganese oxidation. PhD thesis. Rensselaer Polytechnic Inst, Troy, NY.

Arcuri EJ, Ehrlich HL. 1979. Cytochrome involvement in Mn(II) oxidation by two marine bacteria. Appl Environ Microbiol 37:916–923.

Arcuri EJ, Ehrlich HL. 1980. Electron transfer coupled to Mn(II) oxidation in two deep-sea Pacific Ocean isolates. In: Trudinger PA, Walter MR, Ralph BJ, eds. Biogeochemistry of Ancient and Modern Environments. Canberra: Aust Acad Sci, pp 339–344.

Aristovskaya TV. 1961. Accumulation of iron in breakdown of organo-mineral humus complexes by microorganisms. Dokl Akad Nauk SSSR 136:954–957.

Aristovskaya TV, Parinkina OM. 1963. A new soil microorganism *Seliberia stellata* n. gen. n. sp. Akad Nauk SSSR Ser Biol 218:49–56.

Babenko YuS, Dolgikh LM, Serebryanaya MZ. 1983. Characteristics of the bacterial breakdown of primarily oxidized manganese ores of the Nikopol' deposit. Mikrobiologiya 52:851–856.

Baker ET, Massoth GJ. 1986. Hydrothermal plume measurements: A regional perspective. Science 234:980–982.

Balashova VV, Dubinina GA. 1989. The ultrastructure of *Metallogenium* in axenic culture. Mikrobiologiya 58:841–846 (Engl transl pp 681–685).

Bargar JR, Tebo BM, Villinski JE. 2000. In situ characterization of Mn(II) oxidation by spores of the marine *Bacillus* sp. strain SG1. Geochim Cosmochim Acta 64:2775–2778.

Bartlett RJ. 1981. Nonmicrobial nitrite-to-nitrate transformation in soil. Soil Sci Am J 45:1054–1058.

Beijerinck MW. 1913. Oxydation des Mangancarbonates durch Bakterien und Schimmelpilze. Fol Mikrobiol Holländ Beitr Gesamt Mikrobiol Delft 2:123–134.
Bender ML, Ku T.-L. Broecker WS. 1970. Accumulation rates of manganese in pelagic sediments and nodules. Earth Planet Sci Lett 8:143–148.
Beveridge TJ, Kadurugamuwa JL. 1996. Periplasm, periplasmic spaces, and their relation to bacterial wall structure: Novel secretion of selected periplasmic proteins from *Pseudomona aeruginosa*. Microb Drug Resist 2:1–8.
Bignot G, Dangeard L. 1976. Contribution à l'étude de la fraction biogène des nodules polymétalliques des fonds océanique actuels. CR Somm Soc Geol Fr 3:pp 96–99.
Bischoff JL, Rosenbauer RJ. 1983. A note on the chemistry of seawater in the range of 350°–500°C. Geochim Cosmochim Acta 47:139–144.
Boogerd FC, de Vrind JPM. 1987. Manganese oxidation by *Leptothrix discophora*. J Bacteriol 169:489–494.
Bowen HJM. 1966. Trace Elements in Biogeochemistry. London: Academic Press.
Bowen HJM. 1979. Environmental Chemistry of the Elements. London: Academic Press.
Bromfield SM. 1956. Oxidation of manganese by soil microorganisms. Aust J Biol Sci 9:238–252.
Bromfield SM. 1958a. The properties of a biologically formed manganese oxide, its availability to oats and its solution by root washings. Plant Soil 9:325–337.
Bromfield SM. 1958b. The solution of γ-MnO_2 by substances released from soil and from roots of oats and vetch in relation to manganese availability. Plant Soil 10:147–160.
Bromfield SM. 1974. Bacterial oxidation of manganous ion as affected by organic substrate concentration and composition. Soil Biol Biochem 6:383–392.
Bromfield SM. 1978. The oxidation of manganous ions under acid conditions by an acidophilous actinomycete from acid soil. Aust J Soil Res 16:91–100.
Bromfield SM. 1979. Manganous ion oxidation at pH values below 5.0 by cell-free substances from *Streptomyces* sp. cultures. Soil Biol Biochem 11:115–118.
Bromfield SM, David DJ. 1976. Sorption and oxidation of manganous ions and reduction of manganese oxide by cell suspensions of a manganese oxidizing bacterium. Soil Biol Biochem 8:37–43.
Bromfield SM, David DJ. 1978. Properties of biologically formed manganese oxide in relation to soil manganese. Aust J Soil Res 16:79–89.
Bromfield SM, Skerman VDB. 1950. Biological oxidation of manganese in soils. Soil Sci 69:337–348.
Brouwers GJ, de Vrind JPM, Corstjens PLAM, Cornelis P, Baysse C, de Vrind-de Jong EW. 1999. *CumA*, a gene encoding a multicopper oxidase, is involved in Mn^{2+}-oxidation in *Pseudomonas putida* GB-1. Appl Environ Microbiol 65:1762–1768.
Brouwers GJ, Vijgenboom E, Corstjens PLAM, de Vrind JPM, de Vrind-de Jong EW. 2000a. Bacterial Mn^{2+} oxidizing systems and multicopper oxidases: An overview of mechanisms and functions. Geomicrobiol J 17:1–24.
Brouwers GJ, Corstjens PLAM, de Vrind JPM, Verkamman A, De Kuyper M, de Vrind-De Jong EW. 2000b. Stimulation of Mn^{2+} oxidation in *Leptothrix discophora* SS-1 by Cu^{2+} and sequence analysis of the region flanking the gene encoding putative multicopper oxidase MofA. Geomicrobiol J 17:25–33.

Bureau of Mines. 1965. Mineral Facts and Problems. Bull. 630. Washington, DC: Bureau of Mines, US Department of the Interior.

Burdige DJ. 1993. The biogeochemistry of manganese and iron reduction in marine sediments. Earth-Sci Rev 35:249–284.

Burdige DJ, Kepkay PE. 1983. Determination of bacterial manganese oxidation rates in sediments using an in-situ dialysis technique. I. Laboratory studies. Geochim Cosmochim Acta 47:1907–1916.

Burdige DJ, Nealson KH. 1985. Microbiological manganese reduction by enrichment cultures from coastal marine sediments. Appl Environ Microbiol 50:491–497.

Burdige DJ, Nealson KH. 1986. Chemical and microbiological studies of sulfide-mediated manganese reduction. Geomicrobiol J 4:361–378.

Burnett BR, Nealson KH. 1981. Organic films and microorganisms associated with manganese nodules. Deep-Sea Res 28A:637–645.

Burnett BR, Nealson KH. 1983. Energy dispersive X-ray analysis of the surface of a deep-sea ferromanganese nodule. Mar Geol 53:313–329.

Burns RG, Burns VM, Smig W. 1974. Ferromanganese nodule mineralogy: Suggested terminology of the principal manganese oxide phases. Abstr Ann Meet Geol Soc Am.

Butkevich VS. 1928. The formation of marine manganese deposits and the role of microorganisms in the latter. Wissenschaft Meeresinst Ber 3:7–80.

Caccavo F Jr, Blakemore RP, Lovley DR. 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. Appl Environ Microbiol 58:3211–3216.

Caccavo F Jr, Lonergan DJ, Lovley DR, Davis M, Stolz SF, McInerney MJ. 1994. *Geobacter sulfurreducens* sp. nov., a new hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol 60:3752–3759.

Caspi R, Haygood MG, Tebo BM. 1996. Unusual ribulose-1,5-bisphosphate carboxylase/oxygenase genes from a marine manganese oxidizing bacterium. Microbiology (Reading) 142:2549–2559.

Chapnick SD, Moore WS, Nealson KH. 1982. Microbially mediated manganese oxidation in a freshwater lake. Limnol Oceanogr 27:1004–1014.

Clark TR. 1991. Manganese-oxidation by SSW_{22}, a hydrothermal vent bacterial isolate. PhD thesis. Rensselaer Polytechnic Inst, Troy, NY.

Clark TR, Ehrlich HL. 1988. Manganese oxidation by cell fractions from a bacterial hydrothermal vent isolate. Abstr, Annu Meet Am Soc Microbiol K-152, p 232.

Coates JD, Ellis DJ, Gaw CV, Lovley DR. 1999. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer. Int J Syst Bacteriol 49:1615–1622.

Collins BF, Buol SW. 1970. Effects of fluctuations in the E_h-pH environment on iron and/or manganese equilibria. Soil Sci 110:111–118.

Corstjens PLAM, de Vrind JPM, Goosen T, de Vrind-de Jong EW. 1997. Identification and molecular analysis of the *Leptothrix discophora* SS-1 mofA gene, a gene putatively encoding a manganese oxidizing protein with copper domains. Geomicrobiol J 14:91–108.

Cowen JP. 1989. Positive effect on manganese binding by bacteria in deep-sea hydrothermal plumes. Appl Environ Microbiol 55:764–766.

Cowen JP, Bruland KW. 1985. Metal deposits associated with bacteria: Implications for Fe and Mn marine biogeochemistry. Deep-Sea Res 32:253–272.
Cowen JP, Silver MW. 1984. The association of iron and manganese with bacteria on marine microparticulate material. Science 224:1340–1342.
Cowen JP, Massoth GJ, Baker ET. 1986. Bacterial scavenging of Mn and Fe in a mid- to far-field hydrothermal particle plume. Nature (Lond) 322:169–171.
Cowen JP, Massoth GJ, Feely RA. 1990. Scavenging rates of dissolved manganese in a hydrothermal vent plume. Deep-Sea Res 37:1619–1637.
Cowen JP, Bertram MA, Baker ET, Feely RA, Massoth GJ, Summit M. 1998. Geomicrobial transformation of manganese in Gorda Ridge event plumes. Deep-Sea Res II 45:2713–2737.
Crerar DA, Barnes HL. 1974. Deposition of deep-sea manganese nodules. Geochim Cosmochim Acta 38:279–300.
Crerar DA, Fischer AG, Plaza CL. 1979. *Metallogenium* and biogenic deposition of manganese from Precambrian to recent time. In: Geology and Geochemistry of Manganese, Vol III. Budapest: Publ House Hungarian Acad of Sci, pp 285–303.
Cronan DS, Thomas RL. 1970. Ferromanganese concretions in Lake Ontario. Can J Earth Sci 7:1346–1349.
Davison W, Woof C, Rigg E. 1982. The dynamics of iron and manganese in a seasonally anoxic lake: Direct measurement of fluxes using sediment traps. Limnol Oceanogr 27:987–1003.
Dean WE. 1970. Fe-Mn oxidate crusts in Oneida Lake, New York. Proc 13th Conf Great Lakes Res Int Assoc Great Lakes Res, pp 217–226.
Dean WE, Ghosh SK. 1978. Factors contributing to the formation of ferromanganese nodules in Oneida Lake, New York. J Res US Geol Surv 6:231–240.
Dean WE, Greeson PE. 1979. Influences of algae on the formation of freshwater ferromanganese nodules, Oneida Lake, New York. Arch Hydrobiol 86:181–192.
Dean WE, Moore WS, Nealson KH. 1981. Manganese cycles and the origin of manganese nodules, Oneida Lake, New York, U.S.A. Chem Geol 34:53–64.
DePalma SR. 1993. Manganese oxidation by *Pseudomonas putida*. PhD Dissertation, Harvard Univ, Cambridge, MA (as cited by Brouwers et al., 2000a).
de Vrind JPM, Boogerd FC, de Vrind-de Jong EW. 1986a. Manganese reduction by a marine *Bacillus* species. J Bacteriol 167:30–34.
de Vrind JPM, de Vrind-de Jong EW, de Vogt J-WH, Westbroek P, Boogerd FC, Rosson R. 1986b. Manganese oxidation by spore coats of marine *Bacillus* sp. Appl Environ Microbiol 52:1096–1100.
Diem D, Stumm W. 1984. Is dissolved Mn^{2+} being oxidized by O_2 in absence of Mn-bacteria or surface catalysts? Geochim Cosmochim Acta 48:1571–1573.
Di-Ruggiero J, Gounot AM. 1990. Microbial manganese reduction by bacterial strains isolated from aquifer sediments. Microb Ecol 20:53–63.
Dorn RI. 1991. Rock varnish. Am Sci 79:542–553.
Dorn RI, Oberlander TM. 1981. Microbial origin of desert varnish. Science 213:1245–1247.
Douka CE. 1977. Study of bacteria from manganese concretions. Precipitation of manganese by whole cells and cell-free extracts of isolated bacteria. Soil Biol Biochem 9:89–97.

Douka CE. 1980. Kinetics of manganese oxidation by cell-free extracts of bacteria isolated from manganese concretions from soil. Appl Environ Microbiol 39:74–80.

Douka CE, Vaziourakis CD. 1982. Enzymic oxidation of manganese by cell-free extracts of bacteria from manganese concretions from soil. Folia Microbiol 27:418–422.

Drew IM. 1972. Atomic number and periodic table. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 43–48.

Drosdoff M, Nikiforoff CC. 1940. Iron-manganese concretions in Dayton soils. Soil Sci 49:333–345.

Dubinina GA. 1970. Untersuchungen über die Morphologie von *Metallogenium* und die Beziehung zu *Mycoplasma*. Z Allg Mikrobiol 10:309–320.

Dubinina GA. 1978a. Functional role of bivalent iron and manganese oxidation in *Leptothrix pseudoochracea*. Mikrobiologiya 47:783–789 (Engl transl, pp 631–636).

Dubinina GA. 1978b. Mechanism of oxidation of divalent iron and manganese by iron bacteria growing at neutral pH of the medium. Mikrobiologiya 47:591–599 (Engl transl pp 471–478).

Dubinina GA. 1984. Infection of procaryotic and eucaryotic microorganisms with Metallogenium. Curr Microbiol 11:349–356.

Dubinina GA, Deryugina ZP. 1971. Electron microscope study of iron-manganese concretions from Lake Punnus-Yarvi. Dokl Akad Nauk SSSR 201:714–716 (Engl transl, pp 738–740).

Dubinina GA, Zhdanov AV. 1975. Recognition of the iron bacteria "*Siderocapsa*" as *Arthrobacter siderocapsulatus* n. sp. Int. J Syst Bacteriol 25:340–350.

Dugolinsky BK, Margolis SV, Dudley WC. 1977. Biogenic influence on growth of manganese nodules. J Sediment Petrol 47:428–445.

Durand P, Prieur D, Jeanthon C, Jacq E. 1990. Occurrence and activity of heterotrophic manganese oxidizing bacteria associated with Alvinellids (polychaetous annelids) from a deep hydrothermal vent site on the East Pacific Rise. CR Acad Sci (Paris) 310 (ser III):273–278.

Ehrlich HL. 1963. Bacteriology of manganese nodules. I. Bacterial action on manganese in nodule enrichments. Appl Microbiol 11:15–19.

Ehrlich HL. 1964. Microbial transformation of minerals. In: Heukelakian H, Dondero N, eds. Principles and Applications in Aquatic Microbiology. New York: Wiley, pp 43–60.

Ehrlich HL. 1966. Reactions with manganese by bacteria from marine ferromanganese nodules. Dev Ind Microbiol 7:43–60.

Ehrlich HL. 1968. Bacteriology of manganese nodules. II. Manganese oxidation by cell-free extract from a manganese nodule bacterium. Appl Microbiol 16:197–202.

Ehrlich HL. 1972. Response of some activities of ferromanganese nodule bacteria to hydrostatic pressure. In: Colwell RR, Morita RY, eds. Effect of the Ocean Environment on Microbial Activities. Baltimore, MD: Univ Park Press, pp 208–221.

Ehrlich HL. 1973. The biology of ferromanganese nodules. Determination of the effect of storage by freezing on the viable nodule flora, and a check on the reliability of the results from a test to identify MnO_2-reducing cultures. In: Interuniversity Program of Research of Ferromanganese Deposits on the Ocean Floor. Phase I Rep, Seabed Assessment Program, International Decade of Ocean Exploration. Washington, DC: Natl Sci Foundation, pp 217–219.

Ehrlich HL. 1976. Manganese as an energy source for bacteria. In: Environmental Biogeochemistry, Vol 2. Ann Arbor, MI: Ann Arbor Sci Pub, pp 633–644.
Ehrlich HL. 1978a. Inorganic energy sources for chemolithotrophic and mixotrophic bacteria. Geomicrobiol J 1:65–83.
Ehrlich HL. 1978b. Conditions for bacterial participation in the initiation of manganese deposition around marine sediment particles. In: Krumbein WE, ed. Environmental Biogeochemistry and Geomicrobiology, Vol. 3. Methods, Metals, and Assessment. Ann Arbor, MI: Ann Arbor Sci Pub, pp 839–845.
Ehrlich HL. 1980. Bacterial leaching of manganese ores. In: Trudinger PA, Walter MR, Ralph BJ, eds. Biogeochemistry of Ancient and Modern Environments. Canberra, Australia: Austr Acad Sci, pp 609–614.
Ehrlich HL. 1981. Microbial oxidation and reduction of manganese as aids in its migration in soil. In: Colloques Internationaux du C.N.R.S. No. 303: Migrations Organo-Minérales dans les Sols Temperés. Paris: Editions du CNRS, pp 209–213.
Ehrlich HL. 1982. Enhanced removal of Mn^{2+} from seawater by marine sediments and clay minerals in the presence of bacteria. Can J Microbiol 28:1389–1395.
Ehrlich HL. 1983. Manganese-oxidizing bacteria from a hydrothermally active area on the Galapagos Rift. Ecol Bull (Stockh) 35:357–366.
Ehrlich HL. 1984. Different forms of bacterial manganese oxidation. In: Strohl WR, Tuovinen OH, eds. Microbial Chemoautotrophy. Columbus, OH: Ohio State Univ Press, pp 47–56.
Ehrlich HL. 1985. Mesophilic manganese-oxidizing bacteria from hydrothermal discharge areas at 21° north on the East Pacific Rise. In: Caldwell DE, Brierley JA, Brierley CL, eds. Planetary Ecology. New York: Van Nostrand Reinhold, pp 186–194.
Ehrlich HL. 1987. Manganese oxide reduction as a form of anaerobic respiration. Geomicrobiol J 5:423–431.
Ehrlich HL 1988. Bioleaching of manganese by marine bacteria. In: Durand G, Bobichon L, Florent J, eds. Proc, 8th Int Biotechnol Symp, Vol 2. Paris: Soc Franç Microbiol, pp 1094–1105.
Ehrlich HL. 1993a. Electron transfer from acetate to the surface of MnO_2 particles by a marine bacterium. J Ind Microbiol 12:121–128.
Ehrlich HL. 1993b. A possible mechanism for the transfer of reducing power to insoluble mineral oxide in bacterial respiration. In: Torma AE, Apel ML, Brierley CL, eds. Biohydrometallurgical Technologies, Vol II. Warrendale, PA: Min, Metals Mater Soc, pp 415–422.
Ehrlich HL. 1996a. Geomicrobiology. 3rd ed, rev. New York: Marcel Dekker, Chap. 15.
Ehrlich HL. 1996b. How microbes influence mineral growth and dissolution. Chem Geol 132:5–9.
Ehrlich HL. 1999. Microbes as geologic agents: Their role in mineral formation. Geomicrobiol J 16:135–153.
Ehrlich HL. 2000. Ocean manganese nodules: Biogenesis and bioleaching possibilities. Mineral Metallurg Process 17:121–128.
Ehrlich HL, Salerno JC. 1988. Stimulation by ADP and phosphate of Mn^{2+} oxidation by cell extracts and membrane vesicles of induced Mn-oxidizing bacteria. Abstr, Annu Meet Am Soc Microbiol K-151:231.

Ehrlich HL, Salerno JC. 1990. Energy coupling in Mn^{2+} oxidation by a marine bacterium. Arch Microbiol 154:12–17.

Ehrlich HL, Zapkin MA. 1985. Manganese-rich layers in calcareous deposits along the western shore of the Dead Sea may have a bacterial origin. Geomicrobiol J 4:207–221.

Ehrlich HL, Ghiorse WC, Johnson GL II. 1972. Distribution of microbes in manganese nodules from the Atlantic and Pacific Oceans. Dev Ind Microbiol 13:57–65.

Ehrlich HL, Yang SH, Mainwaring JD Jr. 1973. Bacteriology of manganese nodules. VI. Fate of copper, nickel, cobalt and iron during bacterial and chemical reduction of the manganese(IV). Z Allg Mikrobiol 13:39–48.

Ehrlich HL, Graham LA, Salerno JS. 1991. MnO_2 reduction by marine Mn^{2+} oxidizing bacteria from around hydrothermal vents at a mid-ocean spreading center and from the Black Sea. In: Berthelin J, ed. Diversity of Environmental Biogeochemistry. Amsterdam: Elsevier, pp 217–224.

Eleftheriadis DK. 1976. Mangan- und Eisenoxydation in Mineral- und Termal-Quellen-Mikrobiologie, Chemie, und Geochemie. PhD Thesis. Univ Saarlandes, Saarbrücken, West Germany.

Emerson D, Garen RE, Ghiorse WC. 1989. Formation of *Metallogenium*-like structures by a manganese-oxidizing fungus. Arch Microbiol 151:223–231.

Emerson S, Kalhorn S, Jacobs L, Tebo BM, Nealson KH, Rosson RA. 1982. Environmental oxidation rate of manganese(II): Bacterial catalysis. Geochim Cosmochim Acta 46:1073–1079.

Francis CA, Tebo BM. 1999. Marine *Bacillus* spores as catalysts for oxidative precipitation and sorption of metals. J Mol Microbiol Biotechnol 1:71–78.

Francis, CA, Obraztsova AY, Tebo BM. 2000. Dissimilatory metal reduction by the facultative anaerobe *Pantoea agglomerans* SP1. Appl Environ Microbiol 66:543–548.

Fredericks-Jantzen CM, Herman H, Herley P. 1975. Microorganisms on manganese nodules. Nature (Lond) 285:270.

Garey CL, Barber SA. 1952. Evaluation of certain factors involved in increasing manganese availability with sulfur. Soil Sci Soc Am Proc 16:173–175.

Geloso M. 1927. Adsorption au sein des solutions salines par le bioxide de manganèse et généralités sur ce phénomène. III. Adsorption par le bioxide de manganese colloidal. Ann Chim 7:113–150.

Gerretsen FC. 1937. Manganese deficiency of oats and its relation to soil bacteria. Ann Bot 1:207–230.

Ghiorse WC. 1980. Electron microscopic analysis of metal-depositing microorganisms in surface layers of Baltic Sea ferromanganese concretions. In: Truding PA, Walter MR, Ralph BJ, eds. Biogeochemistry of Ancient and Modern Environments. Canberra, Australia: Aust Acad Sci, pp 345–354.

Ghiorse WC. 1984. Biology of iron- and manganese-depositing bacteria. Annu Rev Microbiol 38:515–550.

Ghiorse WC. 1988. Microbial reduction of manganese and iron. In: Zehnder JB, ed. Biology of Anaerobic Microorganisms. New York: Wiley, pp 305–331.

Ghiorse WC, Chapnick SD. 1983. Metal-depositing bacteria and the distribution of manganese and iron in swamp waters. Ecol Bull (Stockh) 35:367–376.

Ghiorse WC, Ehrlich HL. 1976. Electron transport components of the MnO_2 reductase system and the location of the terminal reductase in a marine bacillus. Appl Environ Microbiol 31:977–985.

Ghiorse WC, Ehrlich HL. 1992. Microbial biomineralization of iron and manganese. In: Skinner HCW, Fitzpatrick RW, eds. Biomineralization. Process of Iron and Manganese. Modern and Ancient Environments. Catena Suppl 21. Cremlingen, Germany: Catena Verlag, pp 75–99.

Ghiorse WC, Hirsch P. 1979. An ultrastructural study of iron and manganese deposition associated with extracellular polymers of *Pedomicrobium*-like budding bacteria. Arch Microbiol 123:213–226.

Ghosh SK. 1975. Origin and geochemistry of ferromanganese nodules in Oneida Lake, New York. Diss Abstr Int 36:4905–B.

Ghosh J, Imai K. 1985a. Leaching of manganese dioxide by *Thiobacillus ferrooxidans* growing on elemental sulfur. J Ferment Technol 63:259–262.

Ghosh J, Imai K. 1985b. Leaching mechanism of manganese dioxide by *Thiobacillus ferrooxidans*. J Ferment Technol 63:295–298.

Gillette NJ. 1961. Oneida Lake Pancakes. NY State Conservationist 41(April–May): 21.

Glenn JK, Gold MH. 1985. Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin degrading basidiomycete *Phanerochaete chrysosporium.* Arch Biochem Biophys. 242:329–341.

Glenn JK, Akileswaram L, Gold MH. 1986. Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. Arch Biochem Biophys 251:688–696.

Goldberg ED. 1954. Marine geochemistry. 1. Chemical scavengers of the sea. J Geol 62:249–265.

Goldberg ED, Arrhenius GOS. 1958. Chemistry of Pacific pelagic sediments. Geochim Cosmochim Acta 13:153–212.

Goldschmidt VM. 1954. *Geochemistry.* Oxford, UK: Clarendon Press, pp 621–642.

Gong-Collins E. 1986. A euryhaline, manganese- and iron-oxidizing *Bacilllus megaterium* from a microbial mat at Laguna Figueroa, Baja California, Mexico. Microbios 48:109–126.

Gottfreund J, Schweisfurth R. 1983. Mikrobiologische Oxidation und Reduktion von Manganspecies. Fresenius Z Allg Chem 316:634–638.

Gottfreund J, Schmitt G, Schweisfurth R. 1983. Chemische und mikrobiologische Untersuchungen an Komplexverbindungen des Mangans. Mitteilgn Deutsch. Bodenkundl Gesellsch 38:325–330.

Gottfreund J, Schmitt G, Schweisfurth R. 1985. Wertigkeitswechsel von Manganspecies durch Bakterien in Nährlösungen und in Lockergestein. Landwirtsch Forschung 38:80–86.

Graham JW. 1959. Metabolically induced precipitation of trace elements from sea water. Science 129:1428–1429.

Graham JW, Cooper S. 1959. Biological origin of manganese-rich deposits of the sea floor. Nature (Lond) 183:1050–1051.

Greene AC, Madgwick JC. 1991. Microbial formation of manganese oxides. Appl Environ Microbiol 57:1114–1120.

Greenslate J. 1974a. Manganese and biotic debris associations in some deep-sea sediments. Science 186:529–531.
Greenslate J. 1974b. Microorganisms participate in the construction of manganese nodules. Nature (Lond) 249:181–183.
Gregory E, Perry RS, Staley JT. 1980. Characterization, distribution, and significance of *Metallogenium* in Lake Washington. Microb Ecol 6:125–140.
Hajj J, Makemson J. 1976. Determination of growth of *Sphaerotilus discophorus* in the presence of manganese. Appl Environ Microbiol 32:699–702.
Harada K. 1978. Micropaleontologic investigation of Pacific manganese nodules. Mem Fac Sci Kyoto Univ Ser Geol Mineral 45:111–132.
Hariya Y, Kikuchi T. 1964. Precipitation of manganese by bacteria in mineral springs. Nature (Lond) 202:416–417.
Harriss RC, Troup AG. 1970. Chemistry and origin of freshwater ferromanganese concretions. Limnol Oceanogr 15:702–712.
Hastings D, Emerson S. 1986. Oxidation of manganese by spores of a marine bacillus: Kinetic and thermodynamic considerations. Geochim Cosmochim Acta 50:1819–1824.
Heezen BC, Holister C. 1971. The Face of the Deep. New York: Oxford Univ Press.
Hem JD. 1963. Chemical equilibria and rate of manganese oxidation. US Geol Surv Water Supply Paper 1667-A.
Hem JD. 1981. Rates of manganese oxidation in aqueous systems. Geochim Cosmochim Acta 45:1369–1374.
Hem JD, Lind CJ. 1983. Nonequilibrium models for predicting forms of precipitated manganese oxides. Geochim. Cosmochim Acta 47:2037–2046.
Hem JD, Roberson E, Fournier RB. 1982. Stability of β-MnOOH and manganese oxide deposition from springwater. Water Resources Res 18:563–570.
Herschel A. 1999. Das Verhalten des Mikroorganismus *Metallogenium* spec. in einer kontinuierlichen Kultur. Limnologica 29:105–200.
Heye D, Beiersdorf H. 1973. Radioaktive und magnetische Untersuchungen an Mangan-knollen zur Ermittlung der Wachstumsgeschwindigkeit bzw. zur Altersbestimmung Z Geophysik 39:703–726.
Hobot JA, Carlemalm E, Villigier W, Kellenberger E. 1984. Periplasmic gel: New concept resulting from the reinvestigation of bacterial cell envelope ultrastructure by new methods. J Bacteriol 160:143–152.
Höfer C, Schlosser D. 1999. Novel enzymatic oxidation of Mn^{2+} to Mn^{3+} catalyzed by a fungal laccase. FEBS Lett 451:186–190.
Hogan VC. 1973. Electron transport and manganese oxidation in *Leptothrix discophorus*. PhD Thesis. Ohio State Univ, Columbus, OH.
Holden PJ, Madgwick JC. 1983. Mixed culture bacterial leaching of manganese dioxide. Proc Australas Inst Min Metall 286(June):61–63.
Hood DW. 1963. Chemical oceanography. Oceanogr Mar Biol Annu Rev 1:129–155.
Hood DW, Slowey JF. 1964. Texas A and M Univ Progr Rept, Proj 276, AEC Contract No. AT-(40-1)-2799.
Horn DR, Horn BM, Delach MN. 1972. Distribution of ferromanganese deposits in the world ocean. In: Horn DR, ed. Ferromanganese Deposits on the Ocean Floor. The

Office of the International Decade of Ocean Exploration. Washington, DC: Natl Sci Foundation, pp 9–17.
Hulth S, Aller RC, Gilbert F. 1999. Coupled anoxic nitrification/manganese reduction in marine sediments. Geochim Cosmochim Acta 63:49–66.
Hungate B, Danin A, Pellerin NB, Stemmler J, Kjellander P, Adams BJ, Staley JT. 1987. Characterization of manganese-oxidizing ($Mn^{II} \rightarrow Mn^{IV}$) bacteria from Negev Desert rock varnish: Implications in desert varnish formation. Can J Microbiol 33:939–943.
Imai K, Tano T. 1967. Leaching of manganese by *Thiobacillus thiooxidans*. Hakko Kyokaishi 25:166–167.
Jackson DD. 1901a. A new species of *Crenothrix* (*C. manganifera*). Trans Am Microsc Soc 23:31–39.
Jackson DD. 1901b. The precipitation of iron, manganese, and aluminum by bacterial action. J Soc Chem Ind 21:681–684.
Jacobs L, Emerson S, Skei J. 1985. Partitioning and transport of metals across on O_2/H_2S interface in a permanently anoxic basin: Framvaren Fjord, Norway. Geochim Cosmochim Acta 49:1433–1444.
Jannasch HW. 1984. Microbial processes at deep sea hydrothermal vents. In: Rona PA, Bostrom K, Laubier L, Smith KL, eds. Hydrothermal Processes at Seafloor Spreading Centers. New York: Plenum, pp 677–709.
Jannasch HW, Mottl MJ. 1985. Geomicrobiology of deep-sea hydrothermal vents. Science 229:717–725.
Jannasch HW, Wirsen CO. 1979. Chemosynthetic primary production at East Pacific sea floor spreading centers. BioScience 29:592–598.
Jaquet JM, Nembrini G, Garcia J, Vernet JP. 1982. The manganese cycle in Lac Léman, Switzerland: The role of *Metallogenium*. Hydrobiology 91:323–340.
Jauregui MA, Reisenauer HM. 1982. Dissolution of oxides of manganese and iron by root exudate components. Soil Sci Soc Am J 46:314–317.
Johnson AH, Stokes JL. 1966. Manganese oxidation by *Sphaerotilus discophorus*. J Bacteriol 91:1543–1547.
Johnson D, Chiswell B, O'Halloran K. 1995. Microorganism and manganese cycling in a seasonally stratified freshwater dam. Water Res 29:2739–2745.
Jones CJ, Murray JW. 1985. The geochemistry of manganese in the northeast Pacific Ocean off Washington. Limnol Oceanogr 30:81–92.
Jones JG, Gardener S, Simon BM. 1983. Bacterial reduction of ferric iron in a stratified eutrophic lake. J Gen Microbiol 129:131–139.
Jones JG, Gardener S, Simon BM. 1984. Reduction of ferric iron by heterotrophic bacteria in lake sediments. J Gen Microbiol 130:45–51.
Jung WK, Schweisfurth R. 1979. Manganese oxidation by an intracellular protein of a *Pseudomonas* species. Z Allg Mikrobiol 19:107–115.
Kadko D, Burckle LH. 1980. Manganese nodule growth rates determined by fossil diatom dating. Nature (Lond) 287:725–726.
Kalhorn S, Emerson S. 1984. The oxidation state of manganese in surface sediments of the deep sea. Geochim Cosmochim Acta 48:897–902.
Kalinenko VO, Belokopytova OV, Nikolaeva GG. 1962. Bacteriogenic formation of iron-manganese concretions in the Indian Ocean. Okeanologiya 11:1050–1059 (Engl transl).

Karavaiko GI, Yuchenko VA, Remizov VI, Klyushnikova TM. 1986. Reduction of manganese dioxide by cell-free *Acinetobacter calcoaceticus* extracts. Mikrobiologiya 55:709–714 (Engl transl, pp 553–558).

Kashefi K, Lovley DR. 2000. Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. Appl Environ Microbiol 66:1050–1056.

Kenten RH, Mann PJG. 1949. The oxidation of manganese by plant extracts in the presence of hydrogen peroxide. Biochem J 45:225–263.

Kenten RH, Mann PJG. 1950. The oxidation of manganese by peroxidase systems. Biochem J 50:67–73.

Kepkay PE. 1985a. Kinetics of microbial manganese oxidation and trace metal binding in sediments: Results from an in situ dialysis technique. Limnol Oceanogr 30: 713–726.

Kepkay PE. 1985b. Microbial manganese oxidation and trace metal binding in sediments: Results from an in situ dialysis technique. In: Caldwell DE, Brierley JA, Brierley CL, eds. Planetary Ecology. New York: Van Nostrand Reinhold, pp 195–209.

Kepkay PE. 1985c. Microbial manganese oxidation and nitrification in relation to the occurrence of macrophyte roots in a lacustrine sediment. Hydrobiology 128:135–142.

Kepkay PE, Nealson KH. 1982. Surface enhancement of sporulation and manganese oxidation by a marine bacillus. J Bacteriol 151:1022–1026.

Kepkay PE, Nealson KH. 1987. Growth of a manganese oxidizing *Pseudomonas* sp. in continuous cultures. Arch Microbiol l48:63–67.

Kepkay PE, Burdige DJ, Nealson KH. 1984. Kinetics of bacterial manganese binding and oxidation in a chemostat. Geomicrobiol J 3:245–262.

Kepkay PE, Schwinghamer P, Willar T, Bowen AJ. 1986. Metabolism and metal binding by surface colonizing bacteria: Results of microgradient measurements. Appl Environ Microbiol 51:163–170.

Kessick MA, Vuceta J, Morgan JJ. 1972. Spectrophotometric determination of oxidized manganese with leuco crystal violet. Environ Sci Technol 6:642–644.

Kindle EM. 1932. Lacustrine concretions of manganese. Am J Sci 24:496–504.

Kindle EM. 1935. Manganese concretions in Nova Scotia lakes. Roy Soc Can Trans Sec IV 29:163–180.

Klaveness D. 1977. Morphology, distribution and significance of the manganese-accumulating microorganisms *Metallogenium* in lakes. Hydrobiologia 56:25–33.

Klimov VV. 1984. Charge separation in photosystem II reaction centers. The role of phaeophytin and manganese. In: Sybesina C, ed. Advances in Photosynthetic Research, Vol 1. Proc Int Congr Photosynth 6th, The Hague: Nijhoff, pp 131–138.

Klinkhammer GP, Bender ML. 1980. The distribution of manganese in the Pacific Ocean. Earth Planet Sci Lett 46:361–384.

Kostka, JE, Luther GW III, Nealson, KH. 1995. Chemical and biological reduction of Mn(III) pyrophosphate complexes: Potential importance of dissolved Mn(III) as an environmental oxidant. Geochim Cosmochim Acta 59:885–894.

Kozub JM, Madgwick JC. 1983. Microaerobic microbial manganese dioxide leaching. Proc Australas Inst Min Metall 288(December):51–54.

Krumbein WE. 1969. Über den Einfluss der Mikroflora auf die exogene Dynamik (Verwitterung und Krustenbildung). Geol Rundschau 58:333–365.

Krumbein WE. 1971. Manganese oxidizing fungi and bacteria in recent shelf sediments of the Bay of Biscay and the North Sea. Naturwissenschaften 58:56–57.
Krumbein WE, Altmann HJ. 1973. A new method for the detection and enumeration of manganese-oxidizing and reducing microorganisms. Helgoländer Wissenschaftliche Meeresuntersuchungen 25:347–356.
Krumbein WE, Jens K. 1981. Biogenic rock varnishes of the Negev Desert (Israel): An ecological study of iron and manganese transformation by cyanobacteria and fungi. Oecologia (Berl) 50:25–38.
Ku TL, Broecker WS. 1969. Radiochemical studies on manganese nodules of deep-sea origin. Deep-Sea Res Oceanogr Abstr 16:625–635.
Landing WM, Bruland K. 1980. Manganese in the North Pacific. Earth Planet. Sci Lett 49:45–56.
Langenhoff AAM, Brouwers-Ceiler DL, Engelberting JHL, Quist JJ, Wolkenfeldt JGPN, Zehnder AJB, Schraa G. 1997. Microbial reduction of manganese coupled to toluene oxidation. FEMS Microbiol Ecol 22:119–127.
LaRock PA, Ehrlich HL. 1975. Observations of bacterial microcolonies on the surface of ferromanganese nodules from Blake Plateau by scanning electron microscopy. Microb Ecol 2:84–96.
Larsen I, Little B, Nealson KH, Ray R, Stone A, Tian J. 1998. Manganite reduction by *Shewanella putrefaciens* MR-4. Am Mineral 83:1564–1572.
Laverman AM, Switzer Blum J, Schaefer JK, Phillips EJP, Lovley DR, Oremland RS. 1995. Growth of strain SES-3 with arsenate and other diverse electron acceptors. Appl Environ Microbiol 61:3556–3561.
Lawton K. 1955. Chemical composition of soils. In: Bear FE, ed. Chemistry of the Soil. New York: Reinhold, pp 53–54.
Leeper GW. 1947. The forms and reactions of manganese in the soil. Soil Sci 63:79–94.
Leeper GW, Swaby RJ. 1940. The oxidation of manganous compounds by microorganisms in the soil. Soil Sci 49:163–169.
Letunova SV, Ulubekova MV, Shcherbakov VI. 1978. Manganese concentration by microorganisms inhabiting soils of the manganese biogeochemical province of the Georgian SSR. Mikrobiologiya 47:332–337 (Engl transl, pp 273–278).
Lieske R. 1919. Zur Ernährungsphysiologie der Eisenbakterien. Zentralbl Bakteriol Parasitenk Infektionskr Hyg Abt II 49:413–425.
Logananthan P, Burau RG. 1973. Sorption of heavy metal ions by a hydrous manganese oxide. Geochim Cosmochim Acta 37:1277–1293.
Lovley DR. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol Rev 55: 259–287.
Lovley DR, Goodwin S. 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. Abstr, Annu Meet Am Soc Microbiol NK-30:249.
Lovley DR, Phillips EJP. 1988a. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron and manganese. Appl Environ Microbiol 54:1472–1480.
Lovley DR, Phillips EJP. 1988b. Manganese inhibition of microbial iron reduction in anaerobic sediments. Geomicrobiol J 6:145–155.

Lovley DR, Phillips EJP. 1994a. Novel process for anaerobic sulfate production from elemental sulfur by sulfate-reducing bacteria. Appl Environ Microbiol 60:2394–2399.

Lovley DR, Phillips EJP. 1994b. Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. Appl Environ Microbiol 60:726–728.

Lovley DR, Phillips EJP, Lonergan DJ. 1989. Hydrogen and formate oxidation coupled to dissimilatory reduction of iron and manganese by *Alteromonas putrefaciens*. Appl Environ Microbiol 55:700–706.

Lovley DR, Widman PK, Woodward JC, Phillips JP. 1993a. Reduction of uranium by cytochrome c_3 of *Desulfovibrio vulgaris*. Appl Environ Microbiol 59:3572–3576.

Lovley DR, Giovannoni SJ, White DC, Champine JE, Phillips EJP, Gorby YA, Goodwin S. 1993b. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch Microbiol 159:336–344.

Lower SK, Hochella MF Jr, Beveridge TJ. 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and α-FeOOH. Science 292:1360–1363.

Luther GW III, Sunby B, Lewis BL, Brendel PJ, Silverberg N. 1997. Interactions of manganese with the nitrogen cycle: Alternative pathways to dinitrogen. Geochim Cosmochim Acta 61:4043–4052.

Mahler HR, Cordes EH. 1966. Biological Chemistry. New York: Harper and Row.

Maki JS, Tebo BM, Palmer FE, Nealson KH, Staley JR. 1987. The abundance and biological activity of manganese-oxidizing bacteria and *Metallogenium*-morphotypes in Lake Washington. FEMS Microbiol Ecol 45:21–29.

Mandernack KW. 1992. Oxygen isotopic, mineralogical, and field studies. PhD Thesis. Univ California, San Diego.

Mandernack KW, Tebo BM. 1993. Manganese scavenging and oxidation at hydrothermal vents and in vent plumes. Geochim Cosmochim Acta 57:3907–3923.

Mandernak KW, Post J, Tebo BM. 1995. Manganese mineral formation by bacterial spores of the marine *Bacillus*, strain GS-1: Evidence for the direct oxidation of Mn(II) to Mn(IV). Geochim Cosmochim Acta 59:4393–4408.

Mann PJG, Quastel JH. 1946. Manganese metabolism in soils. Nature (Lond) 158:154–156.

Mann S, Sparks NHC, Scott GHE, de Vrind-de Jong EW. 1988. Oxidation of manganese and formation of Mn_3O_4 (hausmannite) by spore coats of a marine *Bacillus* sp. Appl Environ Microbiol 54:2140–2143.

Margolis JV, Burns RG. 1976. Pacific deep-sea manganese nodules: Their distribution, composition, and origin. Annu Rev Earth Planet Sci 4:229–263.

McKenzie RM. 1967. The sorption of cobalt by manganese minerals in soils. Aust J Soil Res 5:235–246.

McKenzie RM. 1970. The reaction of cobalt with manganese minerals. Aust J Soil Res 8:97–106.

Mercz TI, Madgwick JC. 1982. Enhancement of bacterial manganese leaching by microalgal growth products. Proc Australas Inst Min Metall, 238(September):43–46.

Mero JL. 1962. Ocean-floor manganese nodules. Econ Geol 57:747–767.

Mills VH, Randles CI. 1979. Manganese oxidation in *Sphaerotilus discophorus* particles. J Gen Appl Microbiol 25:205–207.

Mirchink TG, Zaprometova KM, Zvyagintsev DG. 1970. Satellite fungi of manganese oxidizing bacteria. Mikrobiologiya 39:379–383 (Engl transl, pp 327–330).
Moese JR, Brantner H. 1966. Mikrobiologische Studien an manganoxydierenden Bakterien. Zentralbl Bakt Parasitenk Infektionskr Hyg Abt II 120:480–495.
Moffett JW. 1997. The importance of microbial Mn oxidation in the upper ocean: a comparison of the Sargasso Sea and equatorial Pacific. Deep-Sea Res I, 44:1277–1291.
Moffett JW, Ho J. 1996. Oxidation of cobalt and manganese in seawater is a common microbially catalyzed pathway. Geochim Cosmochim Acta 60:3415–3424.
Molisch H. 1910. Die Eisenbakterien. Jena, Germany: Gustav Fischer Verlag.
Moore WS. 1981. Iron-manganese banding in Oneida Lake ferromanganese nodules. Nature (Lond) 292:233–235.
Moore WS, Dean WE, Krishnaswami S, Borole DV. 1980. Growth rates of manganese nodules in Oneida Lake, New York. Earth Planet Sci Lett 46:191–200.
Mulder GE. 1972. Le cycle biologique tellurique et aquatique du fer et du manganèse. Rev Ecol Biol Sol 9:321–348.
Murdoch F, Smith PG. 1999. Formation of manganese micro-nodules on water pipeline materials. Water Res 33:2893–2895.
Murray JW, Balistrieri LS, Paul B. 1984. The oxidation state of manganese in marine sediments and ferromanganese nodules. Geochim Cosmochim Acta 48:1237–1247.
Murray JW, Dillard JG, Giovanoli R, Moers H, Stumm W. 1985. Oxidation of Mn(II): Initial mineralogy, oxidation state and ageing. Geochim Cosmochim Acta 49:463–470.
Mustoe GE. 1981. Bacterial oxidation of manganese and iron in a modern cold spring. Geol Soc Am Bull Part 1, 92:147–153.
Myers CR, Myers JM. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. J Bacteriol 174:3429–3438.
Myers CR, Myers JM. 1993. Ferric reductase is associated with the membranes of *Shewanella putrefaciens* MR-1. FEMS Microbiol Lett 108:15–22.
Myers CR, Myers JM. 1997. Outer membrane cytochromes of *Shewanella putrefaciens* MR-1: Spectral analysis, and purification of the 83-kDa c-type cytochrome. Biochim Biophys Acta 1326:3007–318.
Myers JM, Myers CR. 1998. Isolation and sequence of *omcA*, a gene encoding a decaheme outer membrane cytochrome c of *Shewanella putrefaciens* MR-1, and detection of *omcA* homologs in other strains of *S. putrefaciens*. Biochim Biophys Acta 1873:237–251.
Myers JM, Myers CR. 2000. Role of the tetraheme cytochrome CymA in anaerobic electron transport in cells of *Shewanella putrefaciens* MR-1 with normal levels of menaquinone. J Bacteriol 182:67–75.
Myers JM, Myers CR. 2001. Role for outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. Appl Environ Microbiol 67:260–269.
Myers CR, Nealson KH. 1988a. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science 240:1319–1321.
Myers CR, Nealson KH. 1988b. Microbial reduction of manganese oxides: Interactions with iron and sulfur. Geochim Cosmochim Acta 52:2727–2732.

Myers CR, Alatalo LJ, Myers JM. 1994. Microbial potential for the anaerobic degradation of simple aromatic compounds in sediments of the Milwaukee harbor, Green Bay and Lake Erie. Environ Toxicol Chem 13:461–471.

Nealson KH. 1978. The isolation and characterization of marine bacteria which catalyze manganese oxidation. In: Krumbein WE, ed. Environmental Biogeochemistry, Vol 3. Methods, Metals and Assessment. Ann Arbor, MI: Ann Arbor Sci Publ, pp 847–858.

Nealson KH, Ford J. 1980. Surface enhancement of bacterial manganese oxidation: Implications for aquatic environments. Geomicrobiol J. 2:21–37.

Nealson KH, Saffarini D. 1994. Iron and manganese in anaerobic respiration: Environmental significance, physiology, regulation. Annu Rev Microbiol 48:311–343.

Nealson KH, Tebo BM, Rosson RA. 1988. Occurrence and mechanism of microbial oxidation of manganese. Adv Appl Microbiol 33:279–318.

Neufeld CA. 1904. Z. Unters. Nahrungs-Genussmitt 7:478 (as cited by Moese and Brantner, 1966).

Novozhilova MI, Berezina FS. 1984. Iron- and manganese-oxidizing microorganisms in grounds in the northwestern part of the Indian Ocean and the Red Sea. Mikrobiologiya 53:129–136 (Engl transl, pp 106–112).

Okazaki M, Sugita T, Shimizu M, Ohode Y, Iwamoto K, de Vrind-de Jong EW, de Vrind JPM, Corstjen PLAM. 1997. Partial purification and characterization of manganese oxidizing factors of *Pseudomonas fluorescens* GB-1. Appl Environ Microbiol 63:4793–4799.

Ostwald J. 1981. Evidence for a biogeochemical origin of the Groote Eylandt manganese ores. Econ Geol 76:556–567.

Park PK. 1968. Seawater hydrogen-ion concentration: Vertical distribution. Science 162:357–358.

Paszczynski A, Huynh V-B, Crawford R. 1986. Comparison of lignase-1 and peroxidase-M2 from the white rot fungus *Phanerochaete chrysosporium*. Arch Biochem Biophys 244:750–765.

Patrick WH Jr, Henderson RE. 1981. Reduction and reoxidation cycles of manganese and iron in flooded soil and in water solution. Soil Sci Soc Am J 45:855–859.

Pattan JN, Mudholkar AV. 1990. The oxidation state of manganese in ferromanganese nodules and deep-sea sediments from the Central Indian Ocean. Chem Geol 85:171–181.

Perfil'ev BV, Gabe DR. 1965. The use of the microbial-landscape method to investigate bacteria which concentrate manganese and iron in bottom deposits. In: Perfil'ev BV, Gabe DR, Gal'perina AM, Rabinovich VA, Saponitskii AA, Sherman EE, Troshanov EP, eds. Applied Capillary Microscopy. The Role of Microorganisms in the Formation of Iron Manganese Deposits. New York: Consultants Bureau, pp 9–54.

Perfil'ev BV, Gabe DR. 1969. Capillary Methods of Studying Microorganisms. English translation by J Shewan. Toronto, Canada: Univ Toronto Press.

Perkins EC, Novielli F. 1962. Bacterial leaching of manganese ores. US Bur Mines Rep Invest 6102.

Piper DZ, Basler JR, Bischoff JL. 1984. Oxidation state of marine manganese nodules. Geochim Cosmochim Acta 48:2347–2355.

Poldervaart A. 1955. Chemistry of the Earth's crust. In: Poldervaart A, ed. Crust of the Earth. A Symposium. Special Paper 62 Boulder, CO: Geol Soc Am, pp 119–144.
Potter RM, Rossman GR. 1977. Desert varnish: The importance of clay minerals. Science 196:1446–1448.
Reid ASJ, Miller MH. 1963. The manganese cycle. II. Forms of soil manganese in equilibriuim with solution manganese. Can J Soil Sci 43:250–259.
Reyss JL, Marchig V, Ku TL. 1982. Rapid growth of a deep-sea manganese nodule. Nature (Lond) 295:401–403.
Richards FA. 1965. Anoxic basins and fjords. In: Riley PG, Skirrow G, eds. *Chemical Oceanography.* Vol 1. New York: Academic Press, pp 611–641.
Richardson LL, Aguilar C, Nealson KH. 1988. Manganese oxidation in pH and O_2 environments produced by phytoplankton. Limnol Oceanogr 33:352–363.
Riemann F. 1983. Biological aspects of deep-sea manganese nodule formation. Oceanol Acta 6:303–311.
Robertson LA, Van Niel EWJ, Torremans RAM, Kuenen GJ. 1988. Simultaneous nitrification and denitrification in aerobic chemostat cultures of *Thiosphaera pantotropha*. Appl Environ Microbiol 54:2812–2818.
Robinson WO. 1929. Detection and significance of manganese dioxide in the soil. Soil Sci 27:335–349.
Rossman R, Callender E. 1968. Manganese nodules in Lake Michigan. Science 162:1123–1124.
Rosson RA, Nealson KH. 1982. Manganese binding and oxidation by spores of a marine bacillus. J Bacteriol 151:1027–1034.
Rosson RA, Tebo BM, Nealson KH. 1984. Use of poisons in determination of microbial manganese binding rates in seawater. Appl Environ Microbiol 47:740–745.
Rusin PA, Quintana L, Sinclair NA, Arnold RG, Oden KL. 1991a. Physiology and kinetics of manganese-reducing *Bacillus polymyxa* strain D1 isolated from manganiferous silver ore. Geomicrobiol J 9:13–25.
Rusin PA, Sharp JE, Arnold RG, Sinclair NA. 1991b. Enhanced recovery of manganese and silver from refractory ores. In: Smith RW, Mishra M, eds. *Mineral Bioprocessing*. Warrendale, PA: Mineral, Met Maters Soc, pp 207–218.
Sartory A, Meyer J. 1947. Contribution à l'étude du métabolisme hydrocarboné des bactéries ferrugineuses. CR Acad Sci (Paris) 225:541–542.
Schmidt JM, Sharp WP, Starr MP. 1981. Manganese and iron encrustations and other features of *Planctomyces crassus* Hortobagyi 1965, morphotype Ib of the *Balstocaulis-Planctomyces* group of budding and appendaged bacteria, examined by electron microscopy and X-ray micro-analysis. Curr Microbiol 5:241–246.
Schmidt JM, Sharp WP, Starr MP. 1982. Metallic oxide encrustations of the nonprothecate stalks of naturally occurring populations of *Planctomyces bekfii*. Curr Microbiol 7:389–394.
Schmidt W-D, Overbeck J. 1984. Studies of "iron bacteria" from Lake Pluss (West Germany). 1. Morphology, fine structure and distribution of *Metallogenium* sp. and *Siderocapsa germinata*. Z Allg Mikrobiol 24:329–339.
Schöttle M, Friedman GM. 1971. Fresh water iron-manganese nodules in Lake George, New York. Geol Soc Am Bull 82:101–110.

Schulz-Baldes A, Lewin RA. 1975. Manganese encrustation of zygospores of a *Chlamydomonas* (Chlorophyta: Volvocales). Science 188:1119–1120.

Schweisfurth R. 1968. Untersuchungen über manganoxidierende und -reduzierende Mikroorganismen. Mitt Int Verein Limnol 14:179–186.

Schweisfurth R. 1969. Manganoxidierende Pilze. Zentralbe Bakt Parasitenk Infektionskr Hyg I Abt Orig 212:486–491.

Schweisfurth R. 1971. Manganoxidierende Pilze. I. Vorkommen, Isolierungen und mikroskopische Untersuchungen. Z Allg Mikrobiol 11:415–430.

Schweisfurth R, Hehn G v 1972. Licht- und elektronenmikroskopische Untersuchungen sowie Kulturversuche zum *Metallogenium*-Problem. Zentralbl Bakt Parasitenk Infektionskr Hyg I Abt Orig A220:357–361.

Schweisfurth R, Mertes R. 1962. Mikrobiologische und chemische Untersuchungen über Bildung and Bekämpfung von Manganschlammablagerung einer Druckleitung für Talsperrenwasser. Arch Hyg Bakteriol 146:401–417.

Seyfried WE Jr, Janecky DR. 1985. Heavy metal and sulfur transport during subcritical and supercritical hydrothermal alteration of basalt: Influence of fluid pressure and basalt composition and crystallinity. Geochim Cosmochim Acta 49:2545–2560.

Shanks WC III, Bischoff JL, Rosenbauer RJ. 1981. Seawater sulfate reduction and sulfur isotope fractionation in basaltic systems: Interaction of seawater with fayalite and magnetite at 200–350°C Geochim Cosmochim Acta 45:1977–1995.

Sherman GD, McHargue JS, Hodgkiss WS. 1942. Determination of active manganese in soil. Soil Sci 54:253–257.

Shternberg LE. 1967. Biogenic structures in manganese ores. Mikrobiologiya 36:710–712 (Engl transl, pp 595–597).

Sienko MJ, Plane RA. 1966. Chemistry: Principles and Properties. New York: McGraw-Hill.

Silverio CM. 1985. Microaerobic manganese dioxide reduction. NSTA Technol J, Oct–Dec, pp 51–63.

Silverman MP, Lundgren DG. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans* I. An improved medium and a harvesting procedure for securing high cell yields. J Bacteriol 77:642–647.

Sly LI, Hodkinson MC, Arunpairojana V. 1988. Effect of water velocity on the early development of manganese-depositing biofilm in a drinking-water distribution system. FEMS Microbiol Ecol 53:175–186.

Soehngen NL. 1914. Umwandlung von Manganverbindungen unter dem Einfluss mikrobiologischer Prozesse. Zebtralbl Bakt Parasitk Infektionskr Hyg II Abt 40:545–554.

Sokolova-Dubinina GA, Deryugina ZP. 1967a. On the role of microorganisms in the formation of rhodochrosite in Punnus-Yarvi lake. Mikrobiologiya 36:535-542 (Engl transl, pp 445–451).

Sokolova-Dubinina GA, Deryugina ZP. 1967b. Process of iron-manganese concretion formation in Lake Punnus-Yarvi. Mikrobiologiya 36:1066-1076 (Engl transl, pp 892–900).

Sokolova-Dubinina GA, Deryugina ZP. 1968. Influence of limnetic environmental conditions on microbial manganese ore formation. Mikrobiologiya 37:143–153 (Engl transl, pp 123–128).

Sorem RK, Foster AR. 1972. Internal structure of manganese nodules and implications in beneficiation. In: Horn DR, ed. Ferromanganese deposits on the ocean floor. Washington, DC: Office of the International Decade of Ocean Exploration, Natl Sci Foundation, pp 167–181.

Sorokin YuI. 1971. Microflora of iron-manganese concretions. Mikrobiologiya 40:563–566 (Engl transl, pp 493–495).

Sorokin YuI. 1972. Role of biological factors in the sedimentation of iron, manganese, and cobalt and in the formation of nodules. Oceanology (Oekonologiya) 12:1–11.

Sparrow LA, Uren NC. 1987. Oxidation and reduction of Mn in acid soils: Effect of temperature and soil pH. Soil Biol Biochem 19:143–148.

Stabel HH, Kleiner J. 1983. Endogenic flux of manganese to the bottom of Lake Constance. Arch Hydrobiol 98:307–316

Stone AT. 1987. Microbial metabolites and the reductive dissolution of manganese oxides: Oxalate and pyruvate. Geochim Cosmochim Acta 51:919–925

Sugio T, Katagiri T, Moriyama M, Zhen YL, Inagaki K, Tano T. 1988a. Existence of a new type of sulfide oxidase which utilizes ferric iron as an electron acceptor in *Thiobacillus ferrooxidans.* Appl Environ Microbiol 54:153–157.

Sugio T, Tsujitsu Y, Hirayama K, Inagaki K, Tano T. 1988b. Mechanisms of tetravalent manganese reduction with elemental sulfur. Agric Microbiol Chem 52: 185–190.

Tambiev SB, Demina LS. 1992. Biogeochemistry and fluxes of manganese and some other metals in regions of hydrothermal activities (Axial Mountain, Juan de Fuca Ridge and Guaymas Basin, Gulf of California). Deep-Sea Res 39:687–703.

Taylor RM, McKenzie RM. 1966. The association of trace elements with manganese minerals in Australian soils. Aust J Soil Res 4:29–39.

Taylor RM, McKenzie RM, Norrish K. 1964. The mineralogy and chemistry of manganese in some Australian soils. Aust J Soil Res 2:235–248.

Taylor-George S, Palmer F, Staley JT, Borns DJ, Curtiss B, Adams JB. 1983. Fungi and bacteria involved in desert varnish formation. Microb Ecol 9:227–245.

Tebo BM, Emerson S. 1985. Effect of oxygen tension, Mn(II) concentration, and temperature on the microbially catalyzed Mn(II) oxidation rate in a marine fjord. Appl Environ Microbiol 50:1268–1273.

Tebo BM, Lee Y. 1993. Microbial oxidation of cobalt. In: Torma AE, Wey JE, Lakshmanan VI, eds. Biohydrometallurgical Technologies, Vol 1. Bioleaching Processes. Warrendale, PA: Minerals, Metals Mater Soc, pp 695–704.

Tebo BM, Nealson KH, Emerson S, Jacobs L. 1984. Microbial mediation of Mn(II) and Co(II) precipitation at the O_2/H_2S interfaces in two anoxic fjords. Limnol Oceanogr 29:1247–1258.

Tebo BM, Mandernack K, Rosson RA. 1988. Manganese oxidation by a spore coat or exosporium protein from spores of a manganese(II) oxidizing marine bacillus. Abstr, Annu Meet Am Soc Microbiol I-121:201.

Tebo BM, Ghiorse WC, van Waasbergen LG, Siering PL, Caspi R. 1997. Bacterial mineral formation: Insights into manganese(II) oxidation from molecular genetic and biochemical studies. Rev Mineral 35:225–266.

Ten Khak-mun. 1967. Iron- and manganese-oxidizing microorganisms in soils of South Sakhalin. Mikrobiologiya 36:337–344 (Engl transl, pp 276–281).

Thamdrup B, Finster K, Wuergler Hansen J, Bak F. 1993. Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. Appl Environ Microbiol 59:101–108.

Thamdrup B, Rosselló-Mora R, Amann R. 2000. Microbial manganese and sulfate reduction in Black Sea shelf sediments. Appl Environ Microbiol 66:2888–2897.

Thiel GA. 1925. Manganese precipitated by microorganisms. Econ Geol 20:301–310.

Timonin MI. 1950a. Soil microflora and manganese deficiency. Trans 4th Int Congr Soil Sci 3:97–99.

Timonin MI. 1950b. Soil microflora in relation to manganese deficiency. Sci Agric 30:324–325.

Timonin MI, Illman WI, Hartgerink T. 1972. Oxidation of manganous salts of manganese by soil fungi. Can J Microbiol 18:793–799.

Tipping E, Thompson DW, Davidson W. 1984. Oxidation products of Mn(II) oxidation in lake waters. Chem Geol 44:359–383.

Tipping E, Jones JG, Woof C. 1985. Lacustrine manganese oxides: Mn oxidation states and relationships to "Mn depositing bacteria." Arch Hydrobiol 105:161–175.

Tortoriello RC. 1971. Manganic oxide reduction by microorganisms in fresh water environments. PhD Thesis. Rensselaer Polytechnic Inst, Troy, NY.

Trimble RB. 1968. MnO_2-reduction by two strains of marine ferromanganese nodule bacteria. MS Thesis. Rensselaer Polytechnic Inst, Troy, NY.

Trimble RB, Ehrlich HL. 1968. Bacteriology of manganese nodules. III. Reduction of MnO_2 by two strains of nodule bacteria. Appl Microbiol 16:695–702.

Trimble RB, Ehrlich HL. 1970. Bacteriology of manganese nodules. IV. Induction of an MnO_2-reductase system in a marine *Bacillus*. Appl Microbiol 19:966–972.

Troshanov EP. 1968. Iron- and manganese-reducing microorganisms in ore-containing lakes of the Karelian Isthmus. Mikrobiologiya 37:934–940 (Engl transl, pp 786–791).

Troshanov EP. 1969. Conditions affecting the reduction of iron and manganese by bacteria in the ore-bearing lakes of the Karelian Isthmus. Mikrobiologiya 38:634-643 (Engl transl, pp 528–535).

Trost WR. 1958. The chemistry of manganese deposits. Mines Branch Res Rep R8. Ottawa, Canada: Dept Mines Tech Surveys.

Tuttle JH, Ehrlich HL. 1986. Coexistence of inorganic sulfur metabolism and manganese oxidation in marine bacteria. Abstr, Annu Meet Am Soc Microbiol I-21:168.

Tyler PA. 1970. Hyphomicrobia and the oxidation of manganese in aquatic ecosystems. Antonie v Leeuwenhoek 36:567–578.

Tyler PA, Marshall KC. 1967a. Microbial oxidation of manganese in hydro-electric pipelines. Antonie v Leeuwenhoek 33:171–183.

Tyler PA, Marshall KC. 1967b. Hyphomicrobia: A significant factor in manganese problems. J Am Water Works Assoc 59:1043–1048.

Uren NC, Leeper GW. 1978. Microbial oxidation of divalent manganese. Soil Biol Biochem 10:85–87.

Vandenabeele J, De Beer D, Germonpré R, Van de Sande R, Verstraete W. 1995. Influence of nitrate on manganese removing microbial consortia from sand filters. Water Res 29:579–587.

Van Veen WL. 1972. Factors affecting the oxidation of manganese by *Sphaerotilus discophorus*. Antonie v. Leeuwenhoek 38:623–626.

Van Veen WL. 1973. Biological oxidation of manganese in soils. Antonie v Leeuwenhoek 39:657–662.

Van Waasbergen LG, Hoch JA, Tebo BM. 1993. Genetic analysis of the marine manganese-oxidizing Bacillus sp. SG-1: Protoplast transformation, Tn*917* mutagenesis, and identification of chromosomal loci involved in manganese oxidation. J Bacteriol 175:7594–7603.

Van Waasbergen LG, Hildebrand M, Tebo BM. 1996. Identification and characterization of a gene cluster involved in manganese oxidation by spores of the marine *Bacillus* sp. strain SG-1. J Bacteriol 178:3517–3530.

Varentsov IM. 1972. Geochemical studies on the formation of iron-manganese nodules and crusts in recent basins. I. Eningi-Lampi Lake, Central Karelia. Usta Mineral Petrogr Szeged 20:363–381.

Varentsov IM, Pronina NV. 1973. On the study of mechanisms of iron-manganese ore formation in recent basins: The experimental data on nickel and cobalt. Mineral Depos (Berl) 8:161–178.

Vavra P, Frederick L. 1952. The effect of sulfur oxidation on the availability of manganese. Soil Sci Soc Am Proc 16:141–144.

Vojak PWL, Edwards C, Jones MV. 1984. Manganese oxidation and sporulation by an estuarine *Bacillus* species. Microbios 41:39–47.

Vojak PWL, Edwards C, Jones MV. 1985a. A note on the enumeration of manganese-oxidizing bacteria in estuarine water and sediment samples. J Appl Microbiol 59:375–379.

Vojak PWL, Edwards C, Jones MV. 1985b. Evidence for microbiological manganese oxidation in the River Tamar estuary, South West England. Estuarine Coastal Shelf Sci 20:661–671.

Von Damm KL, Edmond JM, Grant B, Measures CI, Walden B, Weiss RF. 1985. Chemistry of submarine hydrothermal solutions at 21°N, East Pacific Rise. Geochim Cosmochim Acta 49:2197–2220.

von Wolzogen-Kühr CAH. 1927. Manganese in waterworks. J Am Water Works Assoc 18:1–31.

Yurchenko VA, Karavaiko GI, Remizov VI, Klyushnikova TM, Tarusin AD. 1987. Role of the organic acids produced by *Acinetobacter calcoaceticus* in manganese leaching. Prikl Biokhim Mikrobiol 23:404–412.

Zapffe C. 1931. Deposition of manganese. Econ Geol 26:799–832.

Zapkin MA, Ehrlich HL. 1983. A comparison of manganese oxidation by growing and resting cells of a freshwater bacterial isolate, strain FMn1. Z Allg Mikrobiol 23:447–455.

Zavarzin GA. 1961. Symbiotic culture of a new microorganism oxidizing manganese. Microbiologiya 30:393–395 (Engl transl, pp 343–345).

Zavarzin GA. 1962. Symbiotic oxidation of manganese by two species of *Pseudomonas*. Mikrobiologiya 31:586–588 (Engl transl, pp 481–482).

Zavarzin GA. 1964. *Metallogenium symbioticum*. Z Allg Mikrobiol 4:390–395.

Zindulis J, Ehrlich HL. 1983. A novel Mn^{2+}-oxidizing enzyme system in a freshwater bacterium. Z Allg Mikrobiol 23:457–465.

ZoBell CE. 1946. Marine Microbiology. Waltham, MA: Chronica Botanica.

17

Geomicrobial Interactions with Chromium, Molybdenum, Vanadium, Uranium, and Polonium

17.1 MICROBIAL INTERACTIONS WITH CHROMIUM

Occurrence of Chromium

Chromium is not a very plentiful element in the Earth's crust, but it is fairly widespread. Its average crustal abundance of 122 ppm (Fortescue, 1980) is less than that of manganese. Average concentration in rocks ranges from 4 to 90 mg kg^{-1}, in soil around 70 mg kg^{-1}, in freshwater around 1 μg kg^{-1}, and in seawater around 0.3 μg kg^{-1} (Bowen, 1979). Its chief mineral occurrence is as chromite, in which the chromium has an oxidation state of +3. It is a spinel whose end members are $MgCr_2O_4$ and $FeCr_2O_4$. The chromium in the mineral can be partially replaced by Al or Fe. Chromite is of igneous origin. Other chromium minerals of minor occurrence include eskolite (Cr_2O_3), daubréelite ($FeS \cdot Cr_2S_3$), crocoite ($PbCrO_4$), uvarovite, which is also known as garnet [$Ca_3Cr_2(SiO_4)_3$], and others (Smith, 1972).

Chemically and Biologically Important Properties

The element chromium is a member of the first transition series of elements in the periodic table together with scandium, titanium, vanadium, manganese, iron, cobalt, nickel, copper, and zinc. The chief oxidation states of chromium are 0, +2, +3, and +6. A +5 oxidation state is also known, which appears to be of significance in at least some biochemical reductions of Cr(VI) (Shi and Dalal, 1990; Suzuki et al., 1992). The geomicrobially important oxidation states are +3 and +6.

Chromium in the hexavalent state is very toxic, in part because of its high solubility as chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) in the physiological pH range. At high enough concentration, Cr(VI) can be mutagenic and carcinogenic. Chromium in the trivalent state is less toxic, in part probably because it is less soluble in this oxidation state at physiological pH. At neutral pH, Cr^{3+} tends to precipitate as a hydroxide [$Cr(OH)_3$] or a hydrated oxide. Chromate and dichromate are strong oxidizing agents.

Chromium has been reported to be nutritionally essential in trace amounts (Miller and Neathery, 1977; Mertz, 1981). Its metabolic function in biochemical terms is still unclear.

As $Cr_2(SO_4)_3$, K_2CrO_4, or $K_2Cr_2O_7$, chromium is inhibitory to growth of bacteria at appropriate concentrations (e.g., Forsberg, 1978; Wong et al., 1982; Bopp et al., 1983). When taken into a cell, hexavalent chromium can act as a mutagen in prokaryotes and eukaryotes (Nishioka, 1975; Petrelli and DeFlora, 1977; Venitt and Levy, 1974) and as a carcinogen in animals (Gruber and Jennette, 1978; Sittig 1985). In bacteria, chromate can be taken into the cell via the sulfate uptake system, which involves active transport (Ohtake et al., 1987; Silver and Walderhaug, 1992). Whether dichromate is transported by the same system is not clear. Bacterial resistance to Cr(VI) has been observed (e.g., Bader et al., 1999). In some instances, at least, the genetic trait is plasmid-borne (Summers and Jacoby, 1978; Bopp et al., 1983; Cervantes, 1991; Silver and Walderhaug, 1992). In *Pseudomonas fluorescens* LB300, resistance to chromate (CrO_4^{2-}) was found to be due to decreased chromate uptake (Bopp, 1980; Ohtake et al., 1987). In *Pseudomonas ambigua*, chromate resistance was attributed to formation of a thickened cell envelope that reduced permeability of Cr(VI) and to an ability to reduce Cr(VI) to Cr(III) (Horitsu et al., 1987). The basis for resistance to dichromate ($Cr_2O_7^{2-}$) and chromite (Cr^{3+}) has not been clearly established. The resistance mechanism for dichromate need not be the same as for chromate, because *Pseudomonas fluorescens* LB300, which is resistant to chromate, is much more sensitive to dichromate (Bopp, 1980; Bopp et al., 1983). Some bacteria have an ability to accumulate chromium. In at least some cases, the accumulation may be due to adsorption (Coleman and Paran, 1983; Johnson et al., 1981; Marques et al., 1982).

Mobilization of Chromium with Microbially Generated Lixiviants

Thiobacillus thiooxidans and *Thiobacillus ferrooxidans* have been found to solubilize only a limited amount of chromium contained in the mineral chromite (Cr_2O_3) with sulfuric acid generated by the oxidation of sulfur (Ehrlich, 1983). Similarly, acid produced during iron oxidation by *T. ferrooxidans* was able to solubilize only limited amounts of chromium from chromite (Wong et al., 1982). On the other hand, chromium can be successfully leached from some solid industrial wastes with biogenically formed sulfuric acid (Bosecker, 1986).

Bio-oxidation of Chromium(III)

No observations of enzymatic oxidation of Cr(III) to Cr(IV) have been reported. However, nonenzymatic oxidation of Cr(III) to Cr(VI) may occur in soil environments, where biogenic (or abiogenic) Mn(III) or Mn(IV) oxides may oxidize Cr(III) to Cr(VI) (Bartlett and James, 1979). This interaction can be summarized as

$$2Cr^{3+} + 3MnO_2 + 2H_2O \rightarrow 2CrO_4^{2-} + 3Mn^{2+} + 4H^+ \qquad (17.1)$$

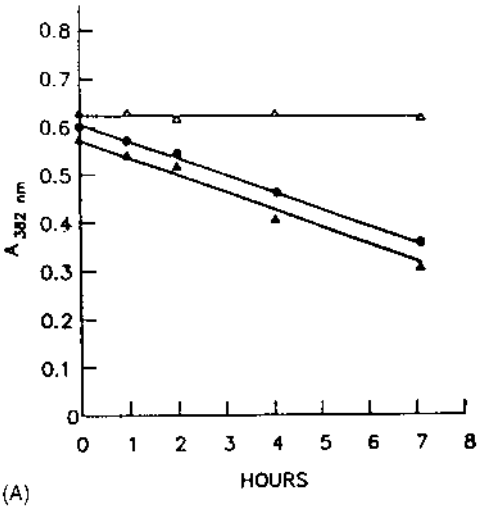
Such oxidation can be detrimental if the Cr(VI) produced reaches a toxic level. Similar observations were made by Chen et al. (1997) and by Kozuh et al. (2000). The latter emphasized that Cr(III) oxidation by Mn(IV) is favored by low organic matter concentration and high concentrations of Mn(IV) oxides.

Bioreduction of Chromium(VI)

Several bacterial species have been shown to reduce Cr(VI) to Cr(III) (Romanenko and Koren'kov, 1977; Horitsu et al., 1978; Lebedeva and Lyalikova, 1979; Shimada, 1979; Kvasnikov et al., 1985; Gvozdyak et al., 1986; Wang et al., 1989; Ishibashi et al., 1990; Shen and Wang, 1993; Philip et al., 1998; Llovera et al., 1993; Lovley and Phillips, 1994; Gopalan and Veeramani, 1994; Garbisu et al., 1998). They include *Achromobacter eurydice*, *Aeromonas dechromatica*, *Agrobacterium radiobacter* strain EPS-916, *Arthrobacter* spp., *Bacillus subtilis*, *B. cereus*, *B. coagulans*, *Desulfovibrio vulgaris* (Hildenborough) ATCC 29579, *Escherichia coli* K-12 and ATCC 33456, *Enterobacter cloacae* HO1, *Flavobacterium devorans*, *Sarcina flava*, *Micrococcus roseus*, *Pseudomonas* spp., and *Shewanella putrefaciens* MR-1. It is unclear whether all these strains reduce Cr(VI) enzymatically. Sulfate-reducing bacteria can reduce chromate with the H_2S they produce from sulfate (Bopp, 1980), but *D. vulgaris* can do it enzymatically as well (Lovley and Phillips, 1994). *T. ferrooxidans* can reduce dichromate with partially reduced sulfur species it forms during the oxidation of elemental sulfur (Sisti et al., 1996). Of those bacteria that reduce Cr(VI)

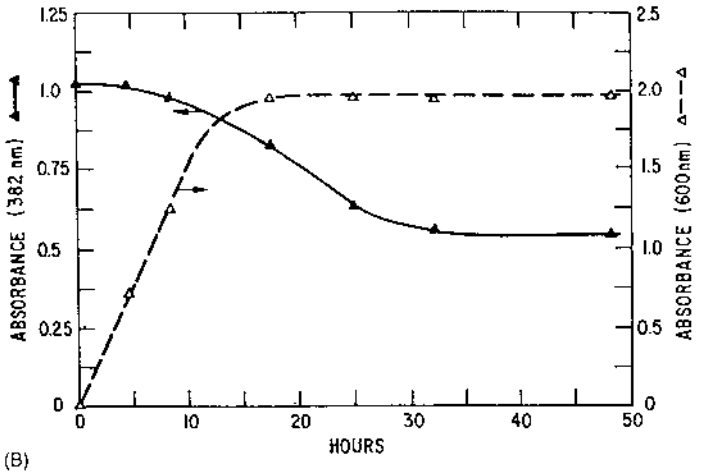
enzymatically, some of the facultative strains reduce it only anaerobically whereas others will do it aerobically and anaerobically. Many bacterial strains reduce Cr(VI) as a form of respiration, but at least one (*P. ambigua* G-1) reduces it as a means of detoxification (Horitsu et al., 1987). Marsh et al. (2000) explored some of the factors that affect biological chromate reduction in microcosms of sandy aquifer material.

P. fluorescens strain LB300, isolated from the upper Hudson River (New York State), can reduce chromate aerobically with glucose or citrate as electron donor (Bopp and Ehrlich, 1988; DeLeo and Ehrlich, 1994). Conditions under which aerobic reduction has been studied include batch culturing with shaking at 200 rpm and continuous culturing with stirring and forced aeration (DeLeo and Ehrlich, 1994). The organism converts chromate to Cr^{3+} in batch culture when growing in glucose–mineral salt solution (Vogel–Bonner medium) and in

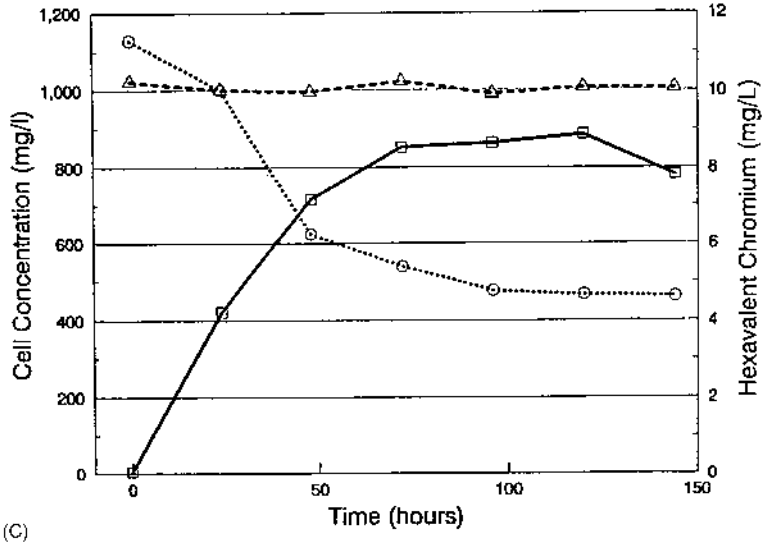


(A)

FIG. 17.1 Chromate reduction by resting and growing cells of chromate-resistant *P. fluorescens* LB300. (A) Resting cells grown with and without chromate. (△) Chromate-grown cells in absence of electron donor (results were the same for cells grown without chromate and assayed in the absence of an electron donor; no chromate reduction was observed); (●) chromate-grown cells with 0.5% (wt/vol) glucose; (▲) cells grown without chromate and assayed with 0.5% (wt/vol) glucose. Chromate was not reduced by spent medium from either chromate-grown cells or cells grown without chromate or by assay buffer containing either 0.25% or 0.5% glucose. Chromate concentration was measured as absorbance at 328 nm after cell removal. [(A) and (B) from Bopp and Ehrlich, 1988, copyright, Springer-Verlag, with permission. (C) from DeLeo and Ehrlich, 1994, copyright Springer-Verlag, with permission.]



(B)



(C)

FIG. 17.1 (*continued*)

continuous culture (chemostat) when growing in a citrate–yeast extract–tryptone solution buffered with phosphate (Fig. 17.1). Anaerobically, *P. fluorescens* strain LB300 was found to reduce chromate only when growing with acetate as energy source (electron donor). Furthermore, whereas aerobically *P. fluorescens* LB300 will reduce chromate at an initial concentration as high as 314 $\mu g\,mL^{-1}$,

anaerobically it reduces chromate only at a concentration below 50 $\mu g\,mL^{-1}$ (Bopp and Ehrlich, 1988; DeLeo and Ehrlich, 1994). Other bacteria that can reduce chromate aerobically and anaerobically include *Escherichia coli* ATCC 33456 (Shen and Wang, 1993) and *Agrobacterium radiobacter* EPS-916 (Llovera et al., 1993). Reduction of chromate by *E. coli* ATCC 33456 is, however, partially repressed by oxygen through uncompetitive inhibition (Shen and Wang, 1993). Reduction of chromate by resting cells of *A. radiobacter* EPS-916 proceeded initially at similar rates aerobically and anaerobically but subsequently slowed significantly in air (Llovera et al., 1994). *Pseudomonas putida* PRS2000 reduces chromate aerobically more rapidly than anaerobically (Ishibashi et al., 1990). *Pseudomonas* sp. strain C7 has so far been tested only aerobically (Gopalan and Veeramani, 1994).

By contrast, *Pseudomonas dechromaticans*, *Pseudomonas chromatophila*, *Enterobacter cloacae* OH1, and *Desulfovibrio vulgaris* reduce Cr(VI) only anaerobically with organic electron donors, or H_2 in the case of *D. vulgaris* (Romanenko and Koren'kov, 1977; Lebededva and Lyalikova, 1979; Komori et al., 1989; Lovley and Phillips, 1994). Except for *E. cloacae* OH1, these organisms cannot use glucose as reductant. *P. dechromaticans* and *P. dechromatophila* appear to be able to reduce both chromate and dichromate.

Cell extracts of *P. fluorescens* LB300 reduce chromate with added glucose or NADH (Fig. 17.2). One or more plasma membrane components appear to be required (Bopp and Ehrlich, 1988). *E. cloacae* HO1 also uses a membrane-bound respiratory system to reduce chromate, but it functions only under anaerobic conditions (Wang et al., 1991). By contrast, most of the chromate-reducing activity in *E. coli* ATCC 33456 appears to be soluble; i.e., it does not involve plasma membrane components, but it is mediated by NADH (Shen and Wang, 1993). The chromate-reducing activity of *P. putida* PRS2000 also does not depend on plasma membrane components. It mediates the reduction via NADH or NADPH (Ishibashi et al., 1990). *D. vulgaris* ATCC 29579 uses its cytochrome c_3 as its Cr(VI) reductase coupled to hydrogenase when using H_2 as reductant (Lovley and Phillips, 1994). In anaerobically grown *Shewanella putrefaciens* MR-1, chromate reductase activity is associated with cytoplasmic membrane (Myers et al., 2000). Although this organism is facultative, it reduces chromate only anaerobically. Both formate and NADH but not L-lactate or NADPH can serve as electron donors to the Cr(VI) reductase system, which includes a multicomponent electron transport system.

Rates of in situ microbial Cr(VI)-reducing activity are generally not readily available, although such measurements would be useful because a number of different bacteria possess the ability to reduce Cr(VI). On the other hand, Wang and Shen (1997) determined rate parameters with a number of pure cultures under laboratory conditions. For example, they reported that the half-maximal Cr(VI)

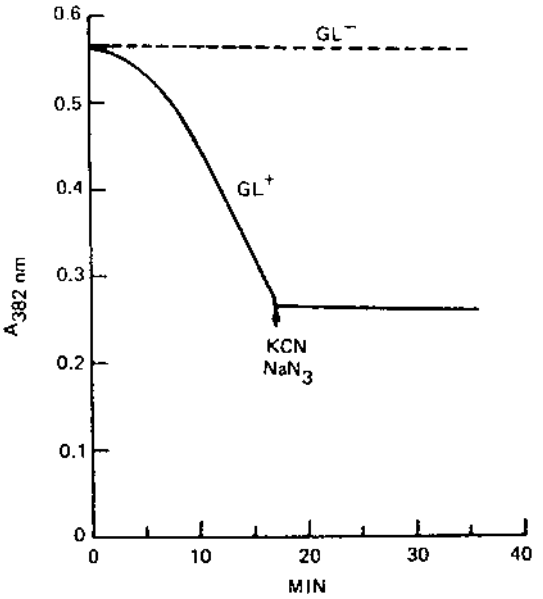


FIG. 17.2 Chromate reduction by cell extract from *P. fluorescens* strain LB300. GL^-, without added glucose; GL^+, with glucose from time 0 and KCN and NaN_3 at times indicated. Chromate concentration measured as absorbance at 382 nm after cell removal. (From Bopp and Ehrlich, 1988, copyright Springer-Verlag, with permission.)

reduction velocity, in mg Cr(VI) L^{-1}, is 5.43 for *B. subtilis* (aerobic), 19.2 for *D. vulgaris* ATCC 29579 (anaerobic), 8.64 for *E. coli* ATCC 33456 (anaerobic), 641.9 for *P. ambigua* G-1 (aerobic), and 5.55 for *P. fluorescens* LB 300 (aerobic), based on Monod kinetics. Natural levels of Cr(VI) in most environments can be expected to be low. However, anthropogenic pollution can cause very significant elevation of environmental chromium concentrations.

Lebedeva and Lyalikova (1979) isolated a strain of *Pseudomonas chromatophila* from the effluent of a chromite mine in Yugoslavia that contained the mineral crocoite ($PbCrO_4$) in its oxidation zone. At this site, the organism clearly found a plentiful source of Cr(VI). The isolate was shown to use a range of carbon and energy sources anaerobically for chromate reduction. These included ribose, fructose, benzoate, lactate, acetate, succinate, butyrate, glycerol, and ethylene glycol but not glucose or hydrogen. Crocoite reduction with lactate as electron donor was demonstrated anaerobically in the laboratory.

Applied Aspects of Chromium(VI) Reduction

The first practical application of microbial Cr(VI) reduction as a bioremediation process was explored by Russian investigators. They presented evidence that indicates that bacterial chromate reduction can be harnessed in waste water and sewage treatment to remove chromate (Romanenko et al., 1976; Pleshakov et al., 1981; Serpokrylov et al., 1985; Simonova et al., 1985). The process also has potential for application in the treatment of tannery and, especially, electroplating wastes and in situ bioremediation. In the case of tannery and electroplating waste treatment, prior dilution of the waste may be necessary to bring the Cr(VI) concentration into a range tolerated by the Cr(VI)-reducing bacteria.

Extensive research in the use of *Enterobacter cloacae* HO1 in bioremediation of Cr(VI)-containing wastewaters was performed in Japan (Ohtake et al., 1990; Komori et al., 1990a, 1990b; Yamamoto et al., 1993; Fujie et al., 1994).

17.2 MICROBIAL INTERACTION WITH MOLYBDENUM

Occurrence and Properties of Molybdenum

Molybdenum is an element of the second transition series in the periodic table. In mineral form, it occurs extensively as molybdenite (MoS_2). The minerals wulfenite ($PbMoO_4$) and powellite ($CaMoO_4$) are often associated with the oxidation zone of molybdenite deposits (Holliday, 1965). Molybdite (MoO_3) is another molybdenum-containing mineral that may be encountered in nature. The abundance of Mo has been reported to be 2–4 g ton^{-1} in basaltic rock, 2.3 g ton^{-1} in granitic rock, and 0.001–0.005 g ton^{-1} in ocean waters (Enzmann, 1972).

The oxidation states in which molybdenum can exist include 0, +2, +3, +4, +5, and +6. Of these, the +4 and +6 states are the most common, but the +5 state is of biological significance. Molybdenum oxyanions of the +6 oxidation state tend to polymerize, the complexity of the polymers depending on the pH of the solution (Latimer and Hildebrand, 1942).

Molybdenum is a biologically important trace element. A number of enzymes feature it in their structure, e.g., nitrogenase, nitrate reductase (Brock and Madigan, 1991), sulfite reductase, and arsenite oxidase (Anderson et al., 1992). Molybdate is an effective inhibitor of bacterial sulfate reduction (Oremland and Capone, 1988).

Microbial Oxidation and Reduction

Molybdenite (MoS_2) is aerobically oxidizable as an energy source by *Acidianus brierleyi* (Brierley and Murr, 1973) (see also Chap. 19) with the formation of molybdate and sulfate. *T. ferrooxidans* can also oxidize molybdenite but is

poisoned by the resulting molybdate (Tuovinen et al., 1971) unless it is rendered insoluble by reaction with Fe^{3+}, for instance. Sugio et al. (1992) reported that *T. ferrooxidans* AP-19-3 contains an enzyme that oxidizes molybdenum blue (Mo^{5+}) to molybdate (Mo^{6+}). They purified the molybdenum oxidase and found it to be an enzyme complex that included cytochrome oxidase as an important component. The function of molybdenum oxidase in the organism remains unclear in view of its sensitivity to molybdate.

Molybdate was first shown to be reduced by *Sulfolobus* sp. by Brierley and Brierley (1982). In a more detailed study, molybdate was shown to be reduced aerobically to molybdenum blue (containing Mo^{5+}) by *Thiobacillus ferrooxidans* using sulfur as electron donor (Sugio et al., 1988). The enzyme that reduced the Mo^{6+} was identified as sulfur : ferric ion oxidoreductase. Molybdate has also been shown to be reduced anaerobically to molybdenum blue by *Enterobacter cloacae* strain 48 using glucose as electron donor (Ghani et al., 1993). The reduction appears to be mediated via NAD and b-type cytochrome.

17.3 MICROBIAL INTERACTION WITH VANADIUM

Occurrence and Properties of Vanadium

Vanadium belongs to the first transition series of elements in the periodic table. In mineral form, it often occurs in complex forms of sulfide, silicate, vanadinite, and uranium vanadate (DeHuff, 1965). Its average abundance in granites is $72\,mg\,kg^{-1}$, in basalts 270, and in soil 90. Its average concentration in freshwater is $0.0005\,ng\,m^{-3}$ and in seawater 0.0025 (Bowen, 1979).

Vanadium occurs in the oxidation states of 0, +2, +3, +4 and +5. Pentavalent vanadium in solution occurs as VO_3^- (vanadate) and is colorless. Tetravalent vanadium is solution occurs as VO^{2+} and is deep blue. Trivalent vanadium (V^{3+}) forms a green solution, and divalent vanadium (V^{2+}) a violet solution (Dickerson et al., 1979).

As a trace element in prokaryotes, vanadium has been found to occur in place of molybdenum in certain nitrogenases (Brock and Madigan, 1991) (see also Chap. 12). It also occurs in oxygen-carrying blood pigment of ascidian worms.

Bacterial Oxidation of Vanadium

Enzymatic oxidation of vanadium compounds by microbes has so far not been reported.

Bacterial Reduction of Vanadium

Five different bacteria have been reported to be able to reduce vanadate. The first three are *Veillonella* (*Micrococcus*) *lactilyticus*, *Desulfovibrio desulfuricans*, and *Clostridium pasteurianum*, which were shown by Woolfolk and Whiteley (1962) to be able to reduce vanadate to vanadyl with hydrogen,

$$VO_3^- + H_2 \rightarrow VO(OH) + OH^- \tag{17.2}$$

The fourth and fifth organisms are new isolates assigned to the genus *Pseudomonas* (Yurkova and Lyalikova, 1990; Lyalikova and Yurkova, 1992). One of these, isolated from a waste stream from a ferrovanadium factory, was named *P. vanadiumreductans*, and the other, isolated from seawater in Kraternaya Bay, Kuril Islands, *P. issachenkovii*. Both are gram-negative, motile, non-spore-forming rods that can grow as facultative chemolithotrophs and facultative anaerobes. Anaerobically, chemolithotrophic growth was observed with H_2 and CO as alternative energy sources, CO_2 as carbon source, and vanadate as terminal electron acceptor. However, the organisms can also grow organotrophically under anaerobic conditions with glucose, maltose, ribose, galactose, lactose, arabinose, lactate, proline, histidine, threonine, and serine as carbon and energy sources. *P. issachenkovii* can also use asparagine as a carbon and energy source. Vanadate reduction involved transformation of pentavalent vanadium to tetravalent and trivalent vanadium. The tetravalent oxidation state was identified in the medium by development of a blue color and the trivalent state by formation of a black precipitate and by its reaction with tairon reagent. An equation describing the overall reduction of vanadium by lactate in these experiments presented by the authors is

$$2NaVO_3 + NaC_3H_5O_3 \rightarrow V_2O_3 + NaC_2H_3O_2 + NaHCO_3 + NaOH \tag{17.3}$$

It accounts for the alkaline pH developed by the medium during growth that started at pH 7.2. Antipov et al. (2000) found that molybdenum- and molybdenum cofactor–free nitrate reductases in *P. issachenkovii* appear to mediate the vanadate reduction. Homogeneous membrane-bound nitrate reductase from the organism reduced vanadate with NADH as electron donor. In a medium containing both nitrate and vanadate, the organism reduced nitrate before vanadate.

A vanadium mineral similar to sherwoodite was detected in cultures reducing vanadium, suggesting that these bacteria may play a role in epigenetic vanadium mineral formation (Lyalikova and Yurkova, 1992).

Vanadium(V) can also be reduced nonenzymatically by bacteria. An example is the reduction of vanadium(V) at concentrations of up to 5 mM to vanadium(IV) by *Thiobacillus thiooxidans*, using elemental sulfur as its energy

source. The vanadium reduction in this instance is brought about by partially reduced sulfur intermediates produced by the organism (Briand et al., 1996).

17.4 MICROBIAL INTERACTION WITH URANIUM

Occurrence and Properties of Uranium

Uranium is one of the naturally occurring radioactive elements. Its abundance in the Earth's crust is only 0.0002%. It is found in more than 150 minerals, but the most important are the igneous minerals pitchblende and coffinite and the secondary mineral carnotite (Baroch, 1965). It is found in small amounts in granitic rocks ($4.4\,mg\,kg^{-1}$) and in even smaller amounts in basalt ($0.43\,mg\,kg^{-1}$). In freshwater it has been reported in concentrations of $0.0004\,mg\,kg^{-1}$ and in seawater, $0.0032\,mg\,kg^{-1}$ (Bowen, 1979).

Uranium can exist in the oxidation states 0, +3, +4, +5, and +6 (Weast and Astle, 1982). The +4 and +6 oxidation states are of greatest significance microbiologically. In nature, the +4 oxidation state usually manifests itself in insoluble forms of uranium, e.g., UO_2. The +6 oxidation state predominates in nature in soluble and hence mobile form, e.g., UO_2^{2+} (Haglund, 1972). In radioactive decay of an isotopic uranium mixture, alpha, beta, and gamma radiation are emitted, but the overall rate of decay is very slow because the dominant isotopes have very long half-lives (Stecher, 1960). This slow rate of decay probably accounts for the ability of bacteria to interact with uranium species without experiencing lethal radiation damage.

Microbial Oxidation of U(IV)

Thiobacillus ferrooxidans has been shown to be able to oxidize tetravalent U^{4+} to hexavalent UO_2^{2+} in a reaction that yields enough energy to enable the organism to fix CO_2. Nevertheless, experimental demonstration of growth of *T. ferrooxidans* with U^{4+} as sole energy source has not succeeded to date. *T. acidophilus* was also found to oxidize U^{4+} but without energy conservation (DiSpirito and Tuovinen, 1981, 1982a, 1982b) (see also Chap. 19).

Microbial Reduction of U(VI)

A number of organisms have been shown to be able to reduce hexavalent uranium (UO_2^{2+}) to tetravalent uranium (UO_2). The first demonstration was with *Veillonella* (*Micrococcus*) *lactilyticus* using H_2 as electron donor under anaerobic conditions (Woolfolk and Whiteley, 1962). Much more recently, some other bacteria were shown to be able to reduce U(VI) to U(IV) anaerobically. They are the facultative organisms *Shewanella putrefaciens* (Lovley et al., 1991) and *S. alga* strain BrY (Caccavo et al., 1992) and the strict anaerobes *Geobacter*

metallireducens strain GS15 (Lovley et al., 1991, 1993a), *Desulfovibrio desulfuricans* (Lovley and Phillips, 1992), *D. vulgaris* (Lovley et al., 1993b), and *Desulfovibrio* sp. (Pietzsch et al., 1999). In *Desulfovibrio*, cytochrome c_3 appears to be the U(VI) reductase (Fig. 17.3) (Lovley et al., 1993b). The electron donors used by these organisms may be organic or, in some instances, H_2. Whereas *G. metallireducens* and *Shewanella putrefaciens* can gain energy from U(VI) reduction (Lovley et al., 1991), *D. desulfuricans* and *D. vulgaris* cannot (Lovley and Phillips, 1992; Lovley et al., 1993c). *Desulfovibrio* sp., on the other hand, is able to gain energy from the process (Pietzsch et al., 1999).

The isolation of some of these organisms from freshwater and marine sediments suggests that this microbial activity may play or may have played a significant role in the immobilization of uranium and its accumulation in sedimentary rock. The first evidence in support of such immobilization was obtained by Gorby and Lovley (1992) in groundwater amended with 0.4 or 1.0 mM uranyl acetate and 30 mM $NaHCO_3$ and inoculated with *Geobacter metallireducens* GS-15, which produced a black precipitate from the dissolved

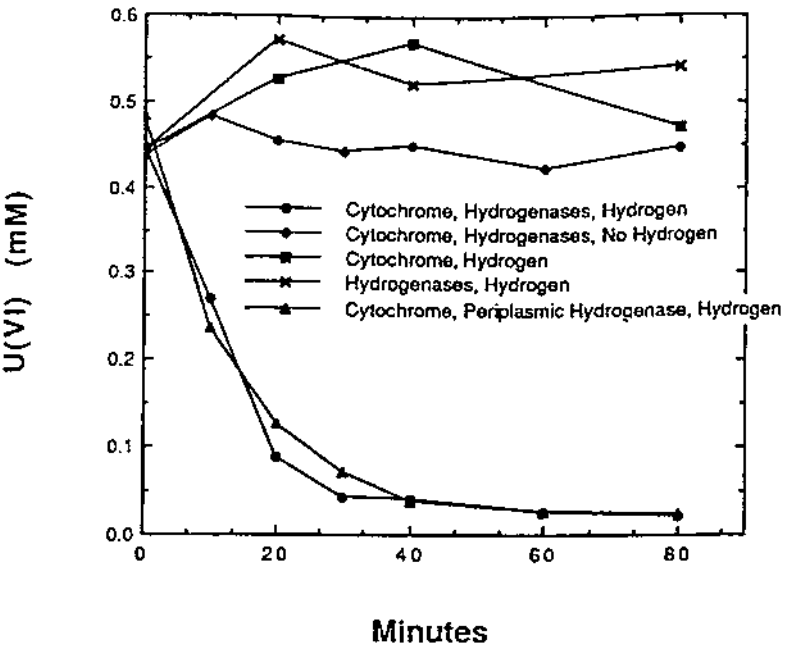


FIG. 17.3 Reduction of U(VI) by electron transfer from H_2 to U(VI) via hydrogenase and cytochrome c_3. As noted, pure periplasmic hydrogenase or a protein fraction containing two hydrogenases was used. (From Lovley et al., 1993b, with permission.)

UO_2^{2+}. The black precipitate was identified as uraninite (UO_2). Frederickson et al. (2000) studied U(VI) reduction by *Shewanella putrefaciens* CN32 in the presence of goethite. They found that besides enzymatic reduction of the U(VI) by *S. putrefaciens* CN32, Fe(II) that had been sorbed to goethite was able to reduce U(VI) abiotically, as was the humic analog anthraquinone-2,6-disulfonate (AQDS).

17.5 BACTERIAL INTERACTION WITH POLONIUM

Polonium is a radioactive element that occurs naturally in association with uranium and thorium minerals. Different isotopes of polonium are produced in the decay of U^{238}, U^{235}, and Th^{232}. Of these isotopes, Po^{210}, which originates from the decay of U^{238}, has the longest half-life (138.4 days) (Lietzke, 1972). Its known oxidation states are +2 and +4 (see LaRock et al., 1996).

Polonium-210 can be an environmental pollutant arising by release from uranium-containing phosphorite, which is commercially exploited for its phosphate, and phosphogypsum, which is a by-product of phosphoric acid manufacture from the phosphorite (see LaRock et al., 1996).

Sulfate-reducing bacteria that were found by LaRock et al. (1996) to be associated with phosphogypsum are able to mobilize Po contained in the phosphogypsum. In laboratory experiments, this mobilization required that the dissolved sulfate concentration in the bulk phase was below 10 μM. Above this sulfate concentration, enough H_2S was produced to coprecipitate the mobilized Po as a metal sulfide (LaRock et al., 1996). The release of polonium apparently depended on the reduction of the sulfate in the gypsum. Aerobic bacteria were also able to mobilize Po in the phosphogypsum. The mechanism in this instance may involve Po complexation by ligands produced by the active organisms (LaRock et al., 1996).

Immobilization of Po by at least one aerobic bacterial isolate has also been reported (Cherrier et al., 1995). The Po was taken into the cell by a mechanism that appeared to differ from that of sulfate uptake. However, its partitioning after uptake paralleled that of sulfur among cell components that included cell envelope, cytoplasm, and cytoplasmic protein. Most polonium and sulfur were detected in the cytoplasmic fraction. In nature, such immobilization of Po must be considered transient if upon death of these cells the Po becomes redissolved in the bulk phase.

17.6 SUMMARY

Enzymatic oxidation of Cr(III) by bacteria has not so far been demonstrated. Nonenzymatic oxidation of Cr(III), which is dependent on biogenic (bacterial,

fungal) formation of Mn(IV), which then oxidizes Cr(III) to Cr(VI) chemically, may occur in soil.

Aerobic and anaerobic reduction of Cr(VI) by bacteria have been demonstrated. The process is in many instances a form of respiration. Various organic electron donors may serve, but not all act equally well aerobically and anaerobically. Chromate and dichromate are not necessarily reduced equally well. The ability to reduce chromate does not always correlate with chromate tolerance.

Although chromite is not very susceptible to leaching by acid formed by acidophiles like *T. ferrooxidans* and *T. thiooxidans*, chromium in some solid inorganic industrial wastes can be leached by sulfuric acid formed by *T. thiooxidans* in sulfur oxidation.

Cr(VI) reduction to Cr(III) is beneficial ecologically because it lowers chromium toxicity. At equivalent concentrations, Cr(VI) is more toxic than Cr(III). Cr(III) also tends to precipitate as a hydroxo compound around neutrality, the pH range at which all known Cr(VI) reducers operate.

Bacteria have been discovered that can enzymatically oxidize Mo(IV) and Mo(V) to Mo(VI) in air. Other bacteria have been found that can enzymatically reduce Mo(VI) to Mo(V), some aerobically, others anaerobically.

Vanadate (VO_3^-) has been found to be reduced anaerobically to vanadyl [VO(OH)] by a number of bacteria. At least two of these organisms can use vanadate as terminal electron acceptor during chemolithotrophic growth with H_2 as electron donor.

Tetravalent uranium can be oxidized to hexavalent uranium, an oxidation that serves as a source of energy to *T. ferrooxidans*, although it does not support its growth as sole energy source. Anaerobically, hexavalent uranium can be reduced to tetravalent uranium by a number of bacteria using either H_2 or one of a variety of organic electron donors.

The bacterial oxidations and reductions of Cr, Mo, V, and U can play an important role in their mobilization or immobilization in soils and sediments.

Polonium can also be mobilized and immobilized by bacteria, but whether redox reactions involving Po are involved remains unknown.

REFERENCES

Anderson GL, Williams J, Hille R. 1992. The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. J Biol Chem 267:23674–23682.

Antipov AN, Lyalikova NN, Khijniak TV, L'vov NP. 2000. Vanadate reduction by molybdenum-free dissimilatory nitrate reductases from vanadate-reducing bacteria. IUBMB Life 50:39–42.

Bader JL, Gonzalez G, Goodell PC. 1999. Chromium-resistant bacterial populations from a site heavily contaminated with hexavalent chromium. Water, Air Soil Pollut 109:263–276.

Baroch CT. 1965. Uranium. In: Mineral Facts and Problems. Bulletin 630. Washington, DC: Bur Mines, US Dept of the Interior, pp 1007–1037.

Bartlett RJ, James BR. 1979. Behavior of chromium in soils: III. Oxidation. J Environ Qual 8:31–35.

Bopp LH. 1980. Chromate resistance and chromate reduction in bacteria. PhD Thesis. Rensselaer Polytechnic Inst, Troy, NY.

Bopp, LH, Ehrlich HL. 1988. Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300. Arch Microbiol 150:426–431.

Bopp LH, Chakrabarty AM, Ehrlich HL. 1983. Chromate resistance plasmid in *Pseudomonas fluorescens*. J Bacteriol 155:1105–1109.

Bosecker K. 1986. Bacterial metal recovery and detoxification of industrial waste. In: Ehrlich HL, Holmes DS, eds. Workshop on Biotechnology for the Mining, Metal-Refining and Fossil Fuel Processing Industries. Biotech Bioeng Symp 16. New York: Wiley, pp 105–120.

Bowen HJM. 1979. Environmental Chemistry of the Elements. London: Academic Press.

Briand L, Thomas H, Donati E. 1996. Vanadium(V) reduction by *Thiobacillus thiooxidans* cultures on elemental sulfur. Biotechnol Lett 18:505–508.

Brierley CL, Brierley JA. 1982. Anaerobic reduction of molybdenum by *Sulfolobus* species. Zentralbl Bakteriol Hyg I Abt Orig C3:289–294.

Brierley CL, Murr LE. 1973. A chemoautotrophic and thermophilic chemoautotrophic microbe. Science 179:488–490.

Brock TD, Madigan MT. 1991. Biology of Microorganisms. 6th ed. Englewood Cliffs, NJ: Prentice Hall.

Caccavo F Jr, Blakemore RP, Lovley DR. 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. Appl Environ Microbiol 58:3211–3216.

Cervantes C. 1991. Bacterial interactions with chromium. Antonie v Leeuwenhoek 59:229–233.

Chen Y, Chen YY, Lin Q, Hu Z, Hu H, Wu J. 1997. Factors affecting Cr(III) oxidation by manganese oxides. Pedosphere 7:185–192.

Cherrier J, Burnett WC, LaRock PA. 1995. Uptake of polonium and sulfur by bacteria. Geomicrobiol J 13:103–115.

Coleman RN, Paran JH. 1983. Accumulation of hexavalent chromium by selected bacteria. Environ Technol Lett 4:149–156.

DeHuff GL. 1965. Vanadium. In: Mineral Facts and Problems. Bull 630. Washington, DC: Bur Mines, US Dept of the Interior, pp 1039–1053.

DeLeo PC, Ehrlich HL. 1994. Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB300 in batch and continuous culture. Appl Microbiol Biotechnol 40:756–759.

Dickerson RE, Greay HB, Haight GP Jr. 1979. Chemical Principles. 3rd ed. Menlo Park, CA: Benjamin/Cummings.

DiSpirito AA, Tuovinen OH. 1981. Oxygen uptake coupled with uranous sulfate oxidation by *Thiobacillus ferrooxidans* and *T. acidophilus*. Geomicrobiol J 2: 275–291.

DiSpirito AA, Tuovinen OH. 1982a. Uranous ion oxidation and carbon dioxide fixation by *Thiobacillus ferrooxidans*. Arch Microbiol 133:28–32.

DiSpirito AA, Tuovinen OH. 1982b. Kinetics of uranous and ferrous iron oxidation by *Thiobacillus ferrooxidans*. Arch Microbiol 133:33–37.

Ehrlich HL. 1983. Leaching chromite ore and sulfide matte with dilute sulfuric acid generated by *Thiobacillus thiooxidans* from sulfur. In: Rossi G, Torma AE, eds. Recent Progress in Biohydrometallurgy. Iglesias, Italy: Assoc Min Sarda, pp 19–42.

Enzmann RD. 1972. Molybdenum: element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 753–759.

Forsberg CW. 1978. Effects of heavy metals and other trace elements on the fermentative activity of the rumen flora and growth of functionally important rumen bacteria. Can J Microbiol 24:298–306.

Fortescue JAC. 1980. Environmental Geochemistry. New York: Springer-Verlag.

Fredrickson JK, Zachara JM, Kennedy DW, Duff MC, Gorby YA, Li S-MW, Krupka KM. 2000. Reduction of U(VI) in goethtite (α-FeOOH) suspensions by a dissimilatory metal-reducing bacterium. Geochim Cosmochim Acta 64:3085–3098.

Fujie K, Tsuchida T, Urano K, Ohtake H. 1994. Development of a bioreactor system for the treatment of chromate wastewater using Enterobacter cloacae HO1. Water Sci Technol 30:235–243.

Garbisu C, Alkorta I, Llama MJ, Serra JL. 1998. Aerobic chromate reduction by *Bacillus subtilis.* Biodegradation 9:133–141.

Ghani B, Takai M, Hisham NZ, Kishimoto N, Ismail AKM, Tano T, Sugio T. 1993. Isolation and characterization of a Mo^{6+}-reducing bacterium. Appl Environ Microbiol 59:1176–1180.

Gopalan R, Veeramani H. 1994. Studies on microbial chromate reduction by *Pseudomonas* sp. in aerobic continuous suspended culture. Biotechnol Bioeng 43:471–476.

Gorby YA, Lovley DR. 1992. Enzymatic uranium precipitation. Environ Sci Technol 26:205–207.

Gruber JE, Jennette KW. 1978. Metabolism of the carcinogen chromate by rat liver microsomes. Biochem Biophys Res Commun 82:700–706.

Gvozdyak PI, Mogilevich NF, Ryl'skii AF, Grishchenko NI. 1986. Reduction of hexavalent chromium by collection strains of bacteria. Mikrobiologiya 55:962–965 (Engl transl, pp 770–773).

Haglund DS. 1972. Uranium: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 1215–1222.

Holliday RW. 1965. Molybdenum. In: Mineral Facts and Problems. Bull. 630. Washington, DC: US Department of the Interior, pp 595–606.

Horitsu H, Nishida H, Kato H, Tomoyeda M. 1978. Isolation of potassium chromate-tolerant bacterium and chromate uptake by the bacterium. Agric Biol Chem 42:2037–2043.

Horitsu H, Futo S, Myazawa Y, Ogai S, Kawai K. 1987. Enzymatic reduction of hexavalent chromium by hexavalent chromium tolerant *Pseudomonas ambigua* G-1. Biol Chem 51:2417–2420.

Ishibashi Y, Cervantes C, Silver S. 1990. Chromium reduction by *Pseudomonas putida*. Appl Environ Microbiol 56:2268–2270.

Johnson I, Flower N, Loutit MW. 1981. Contribution of periphytic bacteria to the concentration of chromium in the crab *Helice crassa*. Microb Ecol 7:245–252.

Komori K, Wang P-C, Toda K, Ohtake H. 1989. Factors affecting chromate reduction in *Enterobacter cloacae* strain HO1. Appl Microbiol Biotechnol 31:567–570.

Komori K, Rivas A, Toda K, Ohtake H. 1990a. Biological removal of toxic chromium using an *Enterobacter cloacae* strain that reduces chromate under anaerobic conditions. Biotechnol Bioeng 35:951–954.

Komori K, Rivas A, Toda K, Ohtake H. 1990b. A method for removal of toxic chromium using dialysis-sac cultures of a chromate reducing strain of *Enterobacter cloacae*. Appl Microbiol Biotechnol 33:117–119.

Kozuh N, Stupar J, Gorenc B. 2000. Reduction and oxidation processes of chromium in soils. Environ Sci Technol 34:112–119.

Kvasnikov EI, Stepnyuk VV, Klyushnikova TM, Serpokrylov NS, Simonova GA, Kasatkina TP, Panchenko LP. 1985. A new chromium-reducing, gram-variable bacterium with mixed type of flagellation. Mikrobiologiya 54:83–88 (Engl transl, pp 69–75).

LaRock PA, Huyn J-H, Boutelle S, Burnett WC, Hull CD. 1996. Bacterial mobilization of polonium. Geochim Cosmochim Acta 60:4321–4328.

Latimer WM, Hildebrand JH. 1942. Reference Book of Inorganic Chemistry. Rev ed. New York: Macmillan, pp 357–360.

Lebedeva EV, Lyalikova NN. 1979. Reduction of crocoite by *Pseudomonas chromatophila* nov. sp. Mikrobiologiya 48:517–522 (Engl transl, pp 405–410).

Lietzke MH. 1972. Polonium: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 962–964.

Llovera S, Bonet R, Simon-Pujol MD, Congregado F. 1993. Chromate reduction by resting cells of *Agrobacterium radiobacter* EPS-916. Appl Environ Micobiol 59:3516–3518.

Lovley DR, Phillips EJP. 1992. Reduction of uranium by *Desulfovibrio desulfuricans*. Appl Environ Microbiol 58:850–856.

Lovley DR, Phillips EJP. 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. Appl Environ Microbiol 60:726–728.

Lovley DR, Phillips EJP, Gorgy YA, Landa ER. 1991. Microbial reduction of uranium. Nature (Lond) 350:413–416.

Lovley DR, Giovannoni SJ, White DC, Champine JE, Phillips EJP, Gorby YA, Goodwin S. 1993a. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch Microbiol 159:336–344.

Lovley DR, Widman PK, Woodward JC, Phillips EJP. 1993b. Reduction of uranium by cytochrome c_3 of *Desulfovibrio vulgaris*. Appl Environ Microbiol 59:3572–3576.

Lovley DR, Roden EE, Phillips EJP, Woodward JC. 1993c. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar Geol 113:41–53.

Lyalikova NN, Yurkova NA. 1992. Role of microorganisms in vanadium concentration and dispersion. Geomicrobiol J 10:15–26.

Marques AM, Espuny Tomas MJ, Congregado F, Simon-Pujol MD. 1982. Accumulation of chromium by *Pseudomonas aeruginosa*. Microbios Lett 21:143–147.

Marsh TL, Leon NM, McInerney MJ. 2000. Physicochemical factors affecting chromate reduction by aquifer materials. Geomicrobiol J 17:291–303.
Mertz W. 1981. The essential trace elements. Science 213:1332–1338.
Miller WJ, Neathery MW. 1977. Newly recognized trace mineral elements and their role in animal nutrition. BioScience 27:674–679.
Myers CR, Carstens BP, Antholine WE, Myers JM. 2000. Chromium(VI) reductase activity is associated with the cytoplasmic membrane of anaerobically grown *Shewanella putrefaciens* MR-1. J Appl Microbiol 88:98–106.
Nishioka H. 1975. Mutagenic activities of metal compounds in bacteria. Mutat Res 31:185–190.
Ohtake H, Cervantes C, Silver S. 1987. Decreased chromate uptake in *Pseudomonas fluorescens* carrying a chromate resistance plasmid. J Bacteriol 169:3853–3856.
Ohtake H, Fuji E, Toda K. 1990. Reduction of toxic chromate in an industrial effluent by use of a chromate-reducing strain of *Enterobacter cloacae*. Environ Technol 11:663–668.
Oremland RS, Capone DG. 1988. Use of "specific" inhibitors in biogeochemistry and microbial ecology. Adv Microb Ecol 10:285–383.
Petrelli FL, DeFlora S. 1977. Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium*. Appl Environ Microbiol 33:805–809.
Philip L, Iyengar L, Venkobachar C. 1998. Cr(VI) reduction by *Bacillus coagulans* isolated from contaminated soils. J Env Eng 124:1165–1170.
Pietzsch K, Hard BC, Babel W. 1999. A *Desulfovibrio* sp. capable of growing by reducing U(VI). J Basic Microbiol 39:365–372.
Pleshakov VD, Koren'kov VN, Zhukov IM, Serpokrylov NS, Lempert IA, Pankrova II. 1981. Biochemical removal of chromium(VI) compounds from wastewaters. USSR Patent SU 835,978, June 7, 1981.
Romanenko VI, Koren'kov VN. 1977. A pure culture of bacteria utilizing chromates and bichromates as hydrogen acceptors in growth under anaerobic conditions. Mikrobiologiya 46:414–417 (Engl transl, pp 329–332).
Romanenko VI, Kuznetsov SI, Koren'kov VN. 1976. Koren'kov method for biological purification of wastewater. USSR Patent SU 521,234, July 15, 1976.
Serpokrylov NS, Zhukov IM, Simonova GA, Kvasnikov EI, Klyushnikova TM, Kasatkina TP, Kostyukov VP. 1985. Biological removal of chromium(VI) compounds from wastewater by anaerobic microorganisms in the presence of an organic substrate. USSR Patent SU 1,198,020, December 1985.
Shen H, Wang Y-T. 1993. Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. Appl Environ Microbiol 59:3771–3777.
Shi X, Dalal NS. 1990. One-electron reduction of chromate by NADPH-dependent glutathione reductase. J Inorg Biochem 40:1–12.
Shimada A. 1979. Effect of sixvalent chromium on growth and enzyme production of chromium-resistant bacteria. Abstr, Annu Meet Am Soc Microbiol Q[H]25:238.
Silver S, Walderhaug M. 1992. Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol Rev 56:195–228.
Simonova GA, Serpokrylov NS, Tokareva LL. 1985. Adaptation characteristics of a culture of *Aeromonas dechromaticans* KS-11 in microbial removal of hexavalent chromium from water. Izv Sev Kavk Nauhn Tsentra Vyssh Shk Estestv Nauki 4:89–91.

Sisti F, Allegretti P, Donati E. 1996. Reduction of dichromate by *Thiobacillus ferrooxidans*. Biotechnol Lett 18:1477–1480.

Sittig M. 1985. Handbook of Toxic and Hazardous Chemicals and Carcinogens. Park Ridge, NJ: Noyes.

Smith CH. 1972. Chromium: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Science. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 167–170.

Stecher PG, ed. 1960. The Merck Index of Chemicals and Drugs. 7th ed. Rahway, NJ: Merck, p 1493.

Sugio T, Tsujita Y, Katagiri T, Inagaki K, Tano T. 1988. Reduction of Mo $^{6+}$ with elemental sulfur by *Thiobacillus ferrooxidans*. J Bacteriol 170:5956–5959.

Sugio T, Hirajama K, Inagaki K, Tanaka H, Tano T. 1992. Molybdenum oxidation by *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58:1768–1771.

Summers AO, Jacoby GA. 1978. Plasmid-determined resistance to boron and chromium compounds in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 13:637–640.

Suzuki T, Miyata N, Horitsu H, Kawai K, Takamizawa K, Tai Y, Okazaki M. 1992. NAD(P)H- dependent chromium(VI) reductase of *Pseudomonas ambigua* G-1: A Cr(V) intermediate is formed during the reduction of Cr(VI) to Cr(III). J Bacteriol 174:5340–5345.

Tuovinen OH, NiemalÄ SI, Gyllenberg HG. 1971. Tolerance of *Thiobacillus ferroxidans* to some metals. Antonie v Leeuwenhoek 37:489–496.

Venitt S, Levy LS. 1974. Mutagenicity of chromates in bacteria and its relevance to carcinogenesis. Nature (Lond) 250:493–495.

Wang Y-T, Shen H. 1997. Modelling C_r(VI) reduction by pure bacterial cultures. Water Res 31:727–732.

Wang P, Mori T, Komori K, Sasatsu M, Toda K, Ohtake H. 1989. Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. Appl Environ Microbiol 55:1665–1669.

Wang P-C, Toda K, Ohtake H, Kusaka I, Yabe I. 1991. Membrane-bound respiratory system of *Enterobacter cloacae* strain HO1 grown anaerobically with chromate. FEMS Microbiol Lett 78:11–16.

Weast RC, Astle MJ. 1982. CRC Handbook of Chemistry and Physics. 63rd ed. Boca Raton, FL: CRC Press, pp B44–B45.

Wong C, Silver M, Kushner DJ. 1982. Effects of chromium and manganese on *Thiobacillus ferrooxidans*. Can J Microbiol 28:536–544.

Woolfolk CA, Whiteley HR. 1962. Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. J Bacteriol 84:647–658.

Yamamoto K, Kato J, Yano I, Ohtake H. 1993. Kinetics and modeling of hexavalent chromium reduction in *Enterobacter cloacae*. Biotechnol Bioeng 41:129–133.

Yurkova NA, Lyalikova NN. 1990. New vanadate-reducing facultative chemolithotrophic bacteria. Mikrobiologiya 59:968–975 (Engl transl, pp 672–677).

18

Geomicrobiology of Sulfur*

18.1 OCCURRENCE OF SULFUR IN THE EARTH'S CRUST

The abundance of sulfur in the Earth's crust has been reported to be around 520 ppm by Goldschmidt (1954) and 880 ppm by Wedepohl (1984). It is thus one of the more common elements in the biosphere. Its concentration in rocks, including igneous and sedimentary rocks, can range from 270 to 2400 ppm (Bowen, 1979). In freshwater, average sulfur concentrations are around 3.7 ppm and in seawater 905 ppm (Bowen, 1979). In field soils in humid, temperate regions, the total sulfur concentration may range from 100 to 1,500 ppm, of which 50–500 ppm is soluble in weak acid or water (Lawton, 1955). Most of the sulfur in soil of pastureland in humid to semiarid climates is organic, whereas that in drier soils is contained in gypsum ($CaSO_4 \cdot 2H_2O$), epsomite ($MgSO_4 \cdot 7H_2O$),

*In this chapter, the old nomenclature of the Thiobacilli is used to minimize confusion when referring to the scientific literature prior to the year 2000. Based on 16S rRNA gene sequence comparison among the thiobacilli, Kelly and Wood (2000) created the following new generic names for the acidophiles among them: *Thiobacillus thiooxidans, T. ferrooxidans,* and *T. caldus* have been reassigned to the genus *Acidithiobacillus*, and *T. albertis* has been renamed *Acidithiobacillus albertensis*; *T. acidophilus* has been renamed *Acidiphilium acidophilum*; *Thiobacillus halophilus* and *T. neapolitanus* have been reassigned to the genus *Halothiobacillus*; *Thiobacillus perometabolis* has been reassigned to the genus *Thiomonas*; and *Thiobacillus tepidarius* has been reassigned to the genus *Thermithiobacillus*.

and in lesser amounts in sphalerite ($ZnSO_4$), chalcopyrite ($CuFeS_2$), and pyrite or marcasite (FeS_2) (Freney, 1967).

18.2 GEOCHEMICALLY IMPORTANT PROPERTIES OF SULFUR

Inorganic sulfur occurs most commonly in the −2, 0, +2, +4, and +6 oxidation states (Roy and Trudinger, 1970) Table 18.1 lists geomicrobially important forms and their various oxidation states. In nature, the −2, 0, and +6 oxidation states are the most common, represented by sulfide, elemental sulfur, and sulfate, respectively. However, in some environments (e.g., chemoclines in aquatic environments, and in some soils and sediments, etc.), some other mixed oxidation states (e.g., thiosulfate, tetrathionate) may also occur, though in lesser amounts.

Some abiotic reactions involving elemental sulfur may have a geomicrobiological impact. For instance, elemental sulfur (usually written S^0, although it is really S_8 because it consists of eight sulfur atoms in a ring) can react reversibly with sulfite to form thiosulfate (Roy and Trudinger, 1970):

$$S^0 + SO_3^{2-} \Leftrightarrow S_2O_3^{2-} \tag{18.1}$$

The forward reaction is favored by neutral to alkaline pH, whereas the back reaction is favored by acid pH. Thiosulfate is very unstable in aqueous solution

TABLE 18.1 Geomicrobially Important Forms of Sulfur and Their Oxide States

Compound	Formula	Oxidation state(s) of sulfur
Sulfide	S^{2-}	−2
Polysulfide	S_n^{2-}	−2, 0
Sulfur[a]	S_8	0
Hyposulfite (dithionite)	$S_2O_4^{2-}$	+3
Sulfite	SO_3^{2-}	+4
Thiosulfate[b]	$S_2O_3^{2-}$	−1, +5
Dithionate	$S_2O_6^{2-}$	+4
Trithionate	$S_3O_6^{2-}$	−2, +6
Tetrathionate	$S_4O_6^{2-}$	−2, +6
Pentathionate	$S_5O_6^{2-}$	−2, +6
Sulfate	SO_4^{2-}	+6

[a] Occurs in an octagonal ring in crystalline form.
[b] Outer sulfur has an oxidation state of −1; the inner sulfur has an oxidation state of +5.

below pH 5. In stable form it can be readily oxidized or reduced by various bacteria.

Elemental sulfur also reacts with sulfide, forming polysulfides (Roy and Trudinger, 1970), a reaction that can play an important role in elemental sulfur metabolism:

$$S_8{}^0 + HS^- \rightarrow HSS_n{}^- + S_{8-n}{}^0 \tag{18.2}$$

The value of n may equal 2, 3, or higher. It is related to the sulfide concentration.

Polythionates, starting with trithionate ($S_3O_6{}^{2-}$), may be viewed as disulfonic acid derivatives of sulfanes (Roy and Trudinger, 1970). They may be formed as by-products in the oxidation of sulfide and sulfur to sulfate and in disproportionation reactions. Polythionates can also be metabolized by some microorganisms.

18.3 BIOLOGICAL IMPORTANCE OF SULFUR

Sulfur is an important element for life. In the cell it is especially important in stabilizing protein structure and in the transfer of hydrogen by enzymes in redox metabolism. For some prokaryotes, reduced forms of sulfur can serve as sources of energy and/or sources of reducing power. Oxidized forms, especially sulfate but also elemental sulfur, can serve other prokaryotes as electron acceptors. It is these oxidation and reduction reactions involving sulfur and sulfur compounds that are especially important geomicrobiologically.

18.4 MINERALIZATION OF ORGANIC SULFUR COMPOUNDS

As part of the carbon cycle, microbes degrade organic sulfur compounds such as the amino acids cysteine, cystine, and methionine, and agar-agar (a sulfuric acid ester of a linear galactan), tyrosine-*O*-sulfate, and so on. Desulfurization is usually the first step in this degradation. Desulfurization of cysteine by bacteria may occur anaerobically by the reaction (Freney, 1967; Roy and Trudinger, 1970)

$$\begin{array}{l} HSCH_2CHCOOH \\ \quad\quad\;\; | \\ \quad\quad\;\; NH_2 \end{array} + H_2O \xrightarrow[\text{desulfhydrase}]{\text{cysteine}} H_2S + NH_3 + CH_3COCOOH \tag{18.3}$$

or by the reaction (Freney, 1967; Roy and Trudinger, 1970)

$$\mathrm{HSCH_2CHCOOH} \underset{-\mathrm{H_2O}}{\overset{+\mathrm{H_2O}}{\rightleftharpoons}} \mathrm{HOCH_2CHCOOH} + \mathrm{H_2S} \quad (18.4)$$

(NH$_2$ on the left; NH$_2$ on the right; serine sulfhydrase)

The sulfur of cysteine may also be released aerobically as sulfate. The reaction in that instance is not completely certain and may differ with different types of organisms (Freney, 1967; Roy and Trudinger, 1970). Although alanine-3-sulfinic acid [$HO_2SCH_2CH(NH_2)COOH$] has been postulated as a key intermediate by some workers, others have questioned it, at least for rat liver mitochondria (Wainer, 1964, 1967).

Methionine is decomposed by extracts of *Clostridium sporogenes* or *Pseudomonas* sp. as follows (Freney, 1967):

$$\text{Methionine} \rightarrow \alpha\text{-ketobutyrate} + \mathrm{CH_3SH} + \mathrm{NH_3} \quad (18.5)$$

18.5 SULFUR ASSIMILATION

Inorganic sulfur is usually assimilated into organic compounds as sulfate by plants and most microorganisms. One possible pathway of assimilation in bacteria is the reduction of sulfate to sulfide and its subsequent reaction with serine to form cysteine, as in *Salmonella typhimurium* (see Freney, 1967, p. 239)*

$$\mathrm{ATP} + \mathrm{SO_4^{2-}} \xrightarrow{\text{ATP sulfurylase}} \mathrm{APS} + \mathrm{PP_i} \quad (18.6)$$

$$\mathrm{ASP} + \mathrm{ATP} \xrightarrow{\text{APS kinase}} \mathrm{PAPS} + \mathrm{ADP} \quad (18.7)$$

$$\mathrm{PAPS} + 2\mathrm{e} \xrightarrow{\text{PAPS reductase}} \mathrm{SO_3^{2-}} + \mathrm{PAP} \quad (18.8)$$

$$\mathrm{SO_3^{2-}} + 7\mathrm{H^+} + 6\mathrm{e} \xrightarrow{\mathrm{SO_3^{2-}\ reductase}} \mathrm{HS^-} + 3\mathrm{H_2O} \quad (18.9)$$

*APS, adenosine 5′-phosphatosulfate; PAPS, adenosine 3′-phosphate-5′-sulfatophosphate; PP_i, inorganic pyrophosphate; PAP, adenosine 3′,5′-diphosphate.

$$HS^- + \text{serine} \xrightarrow{\text{cysteine synthase}} \text{cysteine} + H_2O \tag{18.10}$$

This reaction sequence has also been found in *Bacillus subtilis, Aspergillus niger, Micrococcus aureus*, and *Enterobacter aerogenes* (Roy and Trudinger, 1970). Reaction (18.10) may be replaced by the sequence:

$$\text{Serine} + \text{acetyl} \sim \text{SCoA} \longrightarrow \underset{\substack{\text{CHNH}_2 \\ | \\ \text{COOH} \\ \textit{O}\text{-acetylserine}}}{\text{CH}_2\text{OOCCH}_3} + \text{CoASH} \tag{18.11}$$

$$\textit{O}\text{-Acetylserine} + H_2S \rightarrow \text{cysteine} + \text{acetate} + H^+ \tag{18.12}$$

This latter sequence has been observed in *Escherichia coli* and *Salmonella typhimurium* (Roy and Trudinger, 1970). The reduction of sulfate to "active thiosulfate" and its incorporation into serine to form cysteine is also possible for some organisms, such as *E. coli* (Freney, 1967).

Sulfate reduction occurs not only as part of an *assimilatory* process but also as a *dissimilatory* or *respiratory* process. The latter occurs in the great majority of known instances only in special anaerobic bacteria. The majority of dissimilatory sulfate reducers known to date are members of the domain Bacteria, but at least two sulfate reducers, *Archeoglobus fulgidus* (Stetter et al., 1987; Stetter, 1988) and *A. profundus* (Burggraf et al., 1990), have been shown to belong to the domain Archaea. It is the dissimilatory sulfate reducers that are geomicrobiologically important.

Almost without exception, assimilatory sulfate reduction does not consume more sulfate than is needed for assimilation, so excess sulfide is not produced. The only known exceptions are a strain of *Bacillus megaterium* (Bromfield, 1953) and *Pseudomonas zelinskii* (Shturm, 1948). Unlike dissimilatory sulfate reduction, assimilatory sulfate reduction is thus a form of transitory sulfur immobilization involving small amounts of sulfur per cell; the fixed sulfur is returned to the sulfur cycle upon the death of the organism that assimilated it.

18.6 GEOMICROBIALLY IMPORTANT TYPES OF BACTERIA THAT REACT WITH SULFUR AND SULFUR COMPOUNDS

Oxidizers of Reduced Sulfur

Most geomicrobiologically important microorganisms that oxidize reduced forms of sulfur in relatively large quantities are prokaryotes. They include representa-

tives of the domains Bacteria and Archaea. They comprise aerobes, facultative organisms, and anaerobes and are mostly obligate or facultative autotrophs or mixotrophs. Among the aerobes in the domain Bacteria, one of the most important groups terrestrially is that of the Thiobacillaceae (Table 18.2). This group includes obligate and facultative autotrophs as well as mixotrophs. Among aerobes in the domain Archaea, one of the most widely studied groups consists of the genera *Sulfolobus* and *Acidianus* (Table 18.2). Another group in the domain Bacteria that oxidizes hydrogen sulfide and that is of some importance in freshwater and marine environments is the Beggiatoaceae (Fig. 18.1). Most known members of this group use hydrogen sulfide mixotrophically or heterotrophically. In the latter instance, they employ H_2S oxidation as protection against metabolically produced H_2O_2 in the absence of catalase (Kuenen and Beudeker, 1982; Nelson and Castenholz, 1981), but at least one marine strain, *Beggiatoa alba* MS-81-6, can grow autotrophically (Nelson and Jannasch, 1983). Other hydrogen sulfide oxidizers found in aquatic environments include *Thiovulum* (autotrophic) (e.g., Wirsen and Jannasch, 1978), *Achromatium, Thiothrix, Thio-*

TABLE 18.2 Some Aerobic Sulfur-Oxidizing Bacteria[a]

Autotrophic	Mixotrophic	Heterotrophic
Thiobacillus thioparus	*Thiobacillus intermedius*	*Thiobacillus perometabolis*
Thiobacillus neapolitanus	*Thiobacillus versutus*[e]	*Beggiatoa* spp.
Thiobacillus tepidarius	*Thiobacillus organoparus*	
Thiobacillus caldus	*Pseudomonas* spp.	
Thiobacillus denitrificans[b]		
Thiobacillus novellus[c]		
Thiobacillus thermophilica[c]		
Sulfobacillus thermosulfidooxidans[c]		
Thiobacillus thiooxidans		
Thiobacillus albertis		
Thiobacillus ferrooxidans		
Beggiatoa alba MS-81-6		
Sulfolobus acidocaldarius[d]		
Acidianus brierleyi[d]		
Thermothrix thiopara[c]		

[a] A more complete survey of aerobic sulfur-oxidizing bacteria can be found in Starr et al. (1981), Holt (1989), and Dworkin (2001).
[b] Facultative anaerobe.
[c] Facultative autotroph.
[d] Archeon.
[e] Can also grow autotrophically and heterotrophically.

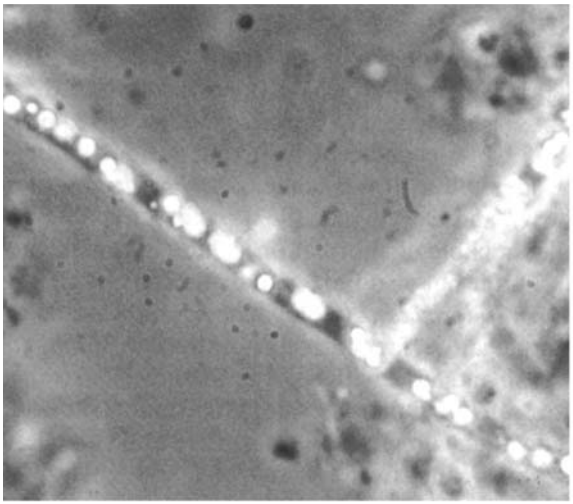


FIG. 18.1 Trichome of *Beggiatoa* with sulfur granules in a pond water enrichment (×5240). (Courtesy of E. J. Arcuri.)

bacterium (La Riviere and Schmidt, 1981), and *Thiomicrospira* (Kuenen and Tuovinen, 1981). Of all these groups, only the thiobacilli produce sulfate directly without accumulating elemental sulfur when oxidizing H_2S at normal oxygen tension. The other groups accumulate sulfur (S^0), which they may oxidize further to sulfate when the supply of H_2S is limited or depleted.

Among members of the domain Bacteria, *Thiobacillus thioparus* oxidizes S^0 slowly to H_2SO_4. It is inhibited as the pH drops below pH 4.5. *T. halophilus* is another neutrophilic, but extremely halophilic, obligate chemolithotroph that oxidizes elemental sulfur to sulfate (Wood and Kelly, 1991). By contrast, *T. thiooxidans, T. albertis* (Bryant et al., 1983), and *T. ferrooxidans* readily oxidize elemental sulfur to sulfuric acid. Being acidophilic, they may drop the pH as low as 1.0 in laboratory culture. All these organisms are strict autotrophs.

The Archaea *Sulfolobus* spp. and *Acidianus* spp. are also able to oxidize sulfur to sulfuric acid. Both genera are extremely thermophilic. *Sulfolobus acidocaldarius* will oxidize sulfur between 55 and 85°C (70–75°C optimum) in a pH range of 0.9–5.8 (pH 2–3 optimum) (Brock et al., 1972; Shivvers and Brock, 1973). The organisms are facultative autotrophs. *Acidianus* (formerly *Sulfolobus*) *brierleyi* has traits similar to those of *S. acidocaldarius* but can also reduce S^0 with H_2 and has a different GC (guanine + cytosine) content (31 mol% versus 37 mol%) (Brierley and Brierley, 1973; Segerer et al., 1986).

Moderately thermophilic bacteria capable of oxidizing sulfur have also been observed. Several are incompletely characterized. Some were isolated from

sulfurous hot springs, others from ore deposits. One of these has been described as a motile rod capable of forming endospores in plectridia. It is a facultative autotroph capable of oxidizing various sulfides and organic compounds besides sulfur. It was named *Thiobacillus thermophilica* Imshenetskii (Egorova and Deryugina, 1963). Another is an aerobic, facultative thermophile capable of sporulation, which is able to oxidize not only elemental sulfur but also Fe^{2+} and metal sulfides. It was named *Sulfobacillus thermosulfidooxidans* (Golovacheva and Karavaiko, 1978). Still another is a gram-negative, facultatively autotrophic *Thiobacillus* sp. capable of growth at 50 and 55°C with a pH optimum of 5.6 (range 4.8–8) (Williams and Hoare, 1972). Other thermophilic thiobacillus-like bacteria have been isolated that can grow on thiosulfate at 60 and 75°C and a pH 7.5, and still others that can grow at 60 and 75°C and at a pH of 4.8 (LeRoux et al., 1977). A moderately thermophilic acidophile, *Thiobacillus caldus*, with an optimum growth temperature of ~ 45°C was isolated by Hallberg and Lindström (1994) and found capable of oxidizing S^{2-}, S^0, SO_3^{2-}, $S_2O_3^{2-}$, and $S_4O_6^{2-}$ (Hallberg et al., 1996).

A number of heterotrophs have been reported to be able to oxidize reduced sulfur in the form of elemental sulfur, thiosulfate, and tetrathionate. They include bacteria and fungi. A diverse group of heterotrophic thiosulfate-oxidizing bacteria have been detected in marine sediments and around hydrothermal vents (Teske et al., 2000). Many bacteria that oxidize elemental sulfur oxidize it to thiosulfate, whereas others oxidize thiosulfate or tetrathionate to sulfuric acid (Guittoneau, 1927; Guittoneau and Keiling, 1927; Grayston and Wainwright, 1988; see also Roy and Trudinger, 1970, pp. 248–249). Some marine pseudomonads and others can gain useful energy from thiosulate oxidation by using it as a supplemental energy source (Tuttle et al., 1974; Tuttle and Ehrlich, 1986).

Two examples of facultatively anaerobic sulfur oxidizers in the domain Bacteria are *Thiobacillus denitrificans* (e.g., Justin and Kelly, 1978) and *Thermothrix thiopara* (Caldwell et al., 1976; Brannan and Caldwell, 1980), the former a mesophile and the latter a thermophile. Anaerobically both organisms use nitrate as terminal electron acceptor and reduce it to oxides of nitrogen and dinitrogen, with nitrite being an intermediate product. They can use sulfur in various oxidation states as energy source. *Thiobacillus denitrificans* is an obligate autotroph whereas *Thermothrix thiopara* is a facultative autotroph.

The strictly anaerobic sulfur oxidizers are represented by photosynthetic purple and green bacteria (Pfennig, 1977) and certain cyanobacteria (Table 18.3). Some of these bacteria may also grow aerobically but without oxidizing reduced sulfur compounds. The purple sulfur bacteria (Chromatiaceae) (Fig. 18.2) are obligate anaerobes that oxidize reduced sulfur, especially H_2S, by using it as a source of reducing power for CO_2 assimilation. Despite the terminology, several purple nonsulfur bacteria (Rhodospirillacea) can also grow autotrophically on H_2S as a source of reducing power for CO_2 assimilation, but for the most part

they tolerate only low concentrations of sulfide, in contrast to purple sulfur bacteria. In the laboratory, purple nonsulfur bacteria can also grow photoheterotrophically, using reduced carbon as a major carbon source. Most purple sulfur bacteria when growing on H_2S oxidize it to S^0, which they deposit *intracellularly* (Fig. 18.2), but *Ectothiorhodospira* spp. are an exception in depositing it extracellularly. Under conditions of H_2S limitation, these strains oxidize the sulfur they accumulate further to sulfate. Among the purple nonsulfur bacteria, *Rhodopseudomonas palustris* and *R. sulfidophila* do not form elemental sulfur as an intermediate from H_2S but oxidize sulfide directly to sulfate (Hansen and van Gemerden, 1972; Hansen and Veldkamp, 1973). In contrast, *Rhodospirillum rubrum, Rhodopseudomonas capsulata*, and *R. spheroides* do form elemental sulfur from sulfide, which they deposit extracellularly (Hansen and van Gemerden, 1972). *R. sulfidophila* differs from most purple nonsulfur bacteria in being more tolerant of a high concentration of sulfide. Green sulfur bacteria (Chlorobiaceae) are strict anaerobic photoautotrophs that oxidize H_2S by using it as a source of reducing power in CO_2 fixation. They deposit the sulfur (S^0) they produce *extracellularly.* Under H_2S limitation, they oxidize the sulfur further to sulfate. At least a few strains of *Chlorobium limicola* forma *thiosulfatophilum* do not accumulate S^0 but oxidize H_2S directly to sulfate (Ivanov, 1968, p. 137; Paschinger et al., 1974). Many photosynthetic anaerobic bacteria can also use thiosulfate as electron donor in place of hydrogen sulfide.

Filamentous, gliding green bacteria (Chloroflexaceae) grow photoheterotrophically under anaerobic conditions, but at least some can also grow photo-

TABLE 18.3 Some Anaerobic Sulfur-Oxidizing Bacteria[a]

Photolithotrophs	Chemolithotrophs
Chromatinum spp.	*Thermothrix thiopara*[b,c]
Chlorobium spp.	*Thiobacillus denitrificans*[c]
Ectothiorhodospira spp.	
Rhodopseudomonas spp.[b]	
Chloroflexus aurantiacus[b]	
Oscillatoria sp.[c]	
Lyngbya spp.[c]	
Aphanothece spp.[c]	
Microcoleus spp.[c]	
Phormidium spp.[c]	

[a] For a more complete description of anaerobic sulfur-oxidizing bacteria, see Starr et al. (1981), Holt (1984), and Dworkin (2001).
[b] Facultatively autotrophic.
[c] Facultatively anaerobic.

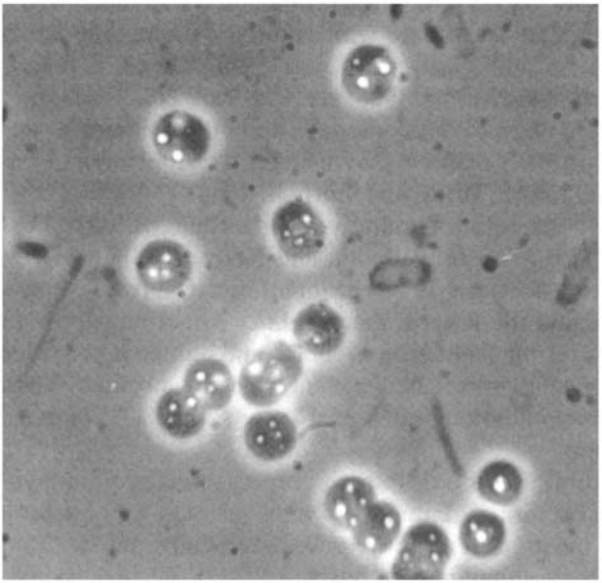


FIG. 18.2 Unidentified purple sulfur bacteria (probably *Chromatium* sp.) in an enrichment culture (×5385). Note the conspicuous sulfur granules in the spherical cells.

autotrophically with H_2S as electron donor under anaerobic conditions (Brock and Madigan, 1988).

A few filamentous cyanobacteria, including some members of the genera *Oscillatoria, Lyngbya, Aphanothece, Microcoleus*, and *Phormidium*, which are normally classified as oxygenic photoautotrophs, can grow photosynthetically under anaerobic conditions with H_2S as a source of reducing power (Cohen et al., 1975; Garlick et al., 1977). They oxidize the H_2S to elemental sulfur and deposit it extracellularly. In the dark, they can re-reduce the sulfur they produce using internal reserves of polyglucose as reductant (Oren and Shilo, 1979). At this time there is no evidence that these organisms can oxidize the sulfur they produce anaerobically further to sulfate under H_2S limitation.

Reducers of Oxidized Sulfur

A geomicrobially and geochemically very important group of bacteria are the sulfate reducers. Most of the known examples belong in the domain Bacteria, but at least two belong to the Archaea, namely *Archeoglobus fulgidus* (Stetter et al., 1987; Speich and Trüper, 1988) and *A. profundus* (Burggraf et al., 1990). More than three decades ago, the sulfate reducers were thought to be represented by

only three genera of the Bacteria, *Desulfovibrio, Desulfotomaculum* (originally classified as *Clostridium* because of its ability to form endospores), and *Desulfomonas*. These organisms are very specialized nutritionally in that among organic energy sources they can use only lactate, pyruvate, fumarate, malate, and ethanol. Furthermore, none of these organisms are able to degrade their organic energy sources beyond acetate (Postgate, 1984), with the result that at the time the importance of the sulfate reducers in anaerobic mineralization of organic matter in sulfate-rich environments was unappreciated. This restricted view of sulfate reducers changed rapidly after 1976 with the discovery of a sulfate reducer, *Desulfotomaculum acetoxidans* (Widdel and Pfennig, 1977, 1981), that is able to oxidize acetate anaerobically to CO_2 and H_2O with sulfate. Subsequently a wide variety of other sulfate reducers were discovered that differed in the nature of the energy sources they were capable of utilizing. Various aliphatic, aromatic, and heterocyclic compounds were found to be attacked and in many cases completely mineralized, each by a specific group of sulfate reducers (e.g., Pfennig et al., 1981; Imhoff-Stuckle and Pfennig, 1983; Braun and Stolp, 1985; Bak and Widdel, 1986a, 1986b; Szewzyk and Pfennig, 1987; Platen et al., 1990; Zellner et al., 1990; Qatabi et al., 1991; Schnell and Schink, 1991; Boopathy and Daniels, 1991; Aeckersberg et al., 1991; Tasaki et al., 1991, 1992; Kuever et al., 1993; Rueter et al., 1994; Rees et al., 1998; Janssen and Schink, 1995; Londry et al., 1999; Meckenstock et al., 2000). Some of the newly discovered sulfate reducers were also found to be able to use H_2 as an energy source. Most require an organic carbon source, but a few can grow autotrophically on hydrogen. Table 18.4 presents a list of some of the different kinds of sulfate reducers in the domain Bacteria and their nutritional ranges. Whereas most sulfate reducers discovered to date are mesophilic, thermophilic types are now also known (e.g., Pfennig et al., 1981; Zeikus et al., 1983; Stetter et al., 1987; Burggraf et al., 1990). Morphologically, sulfate reducers are a very varied group including cocci, sarcinae, rods, vibrios (Fig. 18.3), spirilla, and filaments. The representatives in the domain Bacteria are of a gram-negative cell type.

The two sulfate reducers in the domain Archaea that were discovered by Stetter et al. (1987) and Burggraf et al. (1990) are extremely thermophilic, anaerobic, gram-negative, irregularly shaped cocci. *Archaeoglobus fulgidus* was found to grow naturally in hydrothermal systems at temperatures between 70 and 100°C in the vicinities of Vulcano and Stufe di Nerone, Italy. Under laboratory conditions, the cultures grow anaerobically in marine mineral salts medium supplemented with yeast extract. In this medium they produce a large amount of hydrogen sulfide and some methane. Thiosulfate, but not elemental sulfur, can act as an alternative electron acceptor. Energy sources include hydrogen and some simple organic molecules as well as glucose, yeast extract, and other complex substrates. Cells contain a number of compounds such as 8-OH-5-deazaflavin and methanopterin previously found only in methanogens, which are also members of

TABLE 18.4 Some Sulfate-Reducing Bacteria in the Domain Bacteria[a]

Heterotrophs	Autotrophs[b]
Desulfovibrio desulfuricans[c,d]	*Desulfovibrio baarsii*
Desulfovibrio vulgaris	*Desulfobacter hydrogenophilus*
Desulfovibrio gigas	*Desulfosarcina variabilis*
Desulfovibrio fructosovorans	*Desulfonema limicola*
Desulfovibrio sulfodismutans	
Desulfomonas pigra	
Desulfotomaculum nigrificans	
Desulfotomaculum acetoxidans	
Desulfotomaculum orientis[d]	
Desulfobacter postgatei	
Desulfolobus propionicus	
Desulfobacterium phenolicum[e]	
Desulfobacterium indolicum[f]	
Desulfobacterium catecholicum[g]	

[a] For a more detailed description of sulfate reducers, see Pfennig et al. (1981), Postgate (1984), and Dworkin (2001).
[b] Autotrophic growth on H_2 and CO_2.
[c] Some strains can grow mixotrophically on H_2 and CO_2 + acetate.
[d] At least one strain can grow autotrophically on H_2 and CO_2.
[e] Bak and Widdel (1986b).
[f] Bak and Widdel (1986a).
[g] Szewzyk and Pfennig (1987).

the domain Archaea, but 2-mercaptoethanesulfonic acid and factor F430, which are found in methanogens, were absent (Stetter et al., 1987).

Archoglobus profundus was isolated from the Guaymas hot vent area (Gulf of California, also known as Sea of Cortez). It grows anaerobically at temperatures between 65 and 95°C (optimum 82°C) in a pH range of 4.5–7.5 at an NaCl concentration in the range of 0.9–3.6%. Unlike *A. fulgidus*, it is an obligate mixotroph that requires H_2 as energy source. Its organic carbon requirement can be satisfied by acetate, lactate, pyruvate, yeast extract, meat extract, peptone, or acetate-containing crude oil. As for *A. fulgidus*, sulfate, thiosulfate, and sulfite can serve as electron acceptors for growth. Although S^0 is reduced by resting cells, it inhibits growth (Burggraf et al., 1990).

The presence of other as-yet-unidentified, extremely thermophilic sulfate reducers was detected in hot deep sea sediments at the hydrothermal vents of the tectonic spreading center of the Guaymas Basin (Sea of Cortez or Gulf of California). Sulfate-reducing activity was measurable between 100 and 110°C

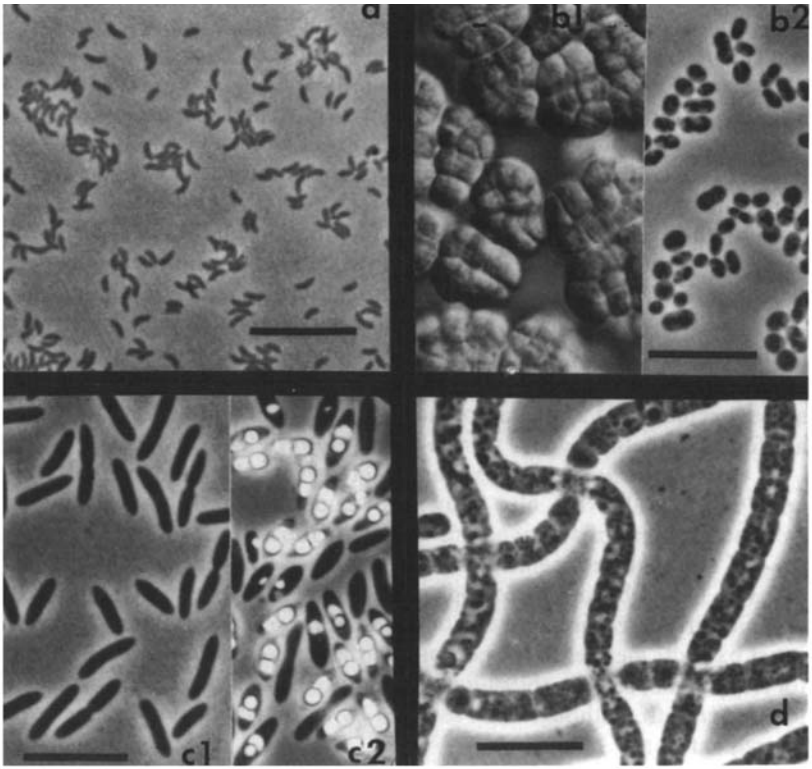


FIG. 18.3 Sulfate-reducing bacteria. (a) *Desulfovibrio desulfuricans* (phase contrast). (b1,b2) *Desulfosarcina variabilis*. (b1) Sarcina packets (interference contrast); (b2) free-living cells (phase contrast). (c1,c2) *Desulfotomaculum acetoxidans*. (c1) Vegetative cells (phase contrast); (c2) cells with spherical spores and gas vacuoles (phase contrast). (d) *Desulfonema limicola* (phase contrast). (From Pfennig N, Widdel F, Trüper HG. 1981. The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, eds. The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, Vol. 1. Copyright Springer-Verlag, Berlin, with permission.)

(optimum 103–106°C). The responsible organisms are probably examples of Archaea (Jørgensen et al., 1992).

Dissimilatory sulfate-reducing activity is not restricted to mesophiles and thermophiles. At least one moderate psychrophile (growth optimum 18–19°C) has been described (Isaksen and Teske, 1996). It has been named *Desulforhopalus vacuolatus* gen. nov., spec. nov. It was isolated from sediment in Kysing Fjord, Denmark, at 10°C. It grows anaerobically on propionate, lactate, and alcohols as energy and carbon sources with sulfate as electron acceptor. It also can grow heterotrophically with H_2 as energy source. Its cells are vacuolated.

Some Clostridia have been found to be strong sulfite reducers. They cannot reduce sulfate in a dissimilatory mode, however. An example is *Clostridium pasteurianum* (McCready et al., 1975; Laishley and Krouse, 1978). The geomicrobial significance of this trait is not clear because sulfite normally does not occur free in the environment in significant amounts in the absence of pollution due to human activities. An exception may occur around volcanic vents that may emit SO_2. This gas, which may also be formed when sulfur-containing fossil fuels are combusted, forms H_2SO_3 when it dissolves in water. *Shewanella putrefaciens* from the Black Sea is another organism that cannot reduce sulfate but can reduce thiosulfate, sulfite, and elemental sulfur to sulfide (Perry et al., 1993). The organism represents 20–50% of the bacterial population in the suboxic zone of the Black Sea, where its ability to reduce partially oxidized sulfur compounds appears to play a significant role in the sulfur cycle.

A number of different bacteria have been found to be able to use elemental sulfur anaerobically as terminal electron acceptor, reducing the sulfur to H_2S (Pfennig and Biebl, 1981). Some of these bacteria can grow autotrophically on sulfur using H_2 or methane as energy sources. They are generally thermophilic members of the domain Archaea (e.g., Stetter et al., 1983; Segerer et al., 1986). Others grow heterotrophically on elemental sulfur, using organic carbon as complex as sugars or as simple as acetate as electron donors (Belkin et al., 1986; Pfennig and Biebl, 1976) to reduce the sulfur in their energy metabolism. This group includes members of both the Bacteria and the Archaea. A few, but by no means all, strains of sulfate-reducing bacteria of the genus *Desulfovibrio* have the ability to use sulfur in place of sulfate as terminal electron acceptor for growth, but strains of *Desulfotomaculum* and *Desulfomonas* are unable to do so (Biebl and Pfennig, 1977).

Some fungi can also reduce sulfur to sulfide with an electron donor such as glucose (e.g., Ehrlich and Fox, 1967). As expected, the activity is greater anaerobically than aerobically.

18.7 PHYSIOLOGY AND BIOCHEMISTRY OF MICROBIAL OXIDATION OF REDUCED FORMS OF SULFUR

Sulfide

Aerobic Attack

Many aerobic bacteria that oxidize sulfide are obligate or facultative chemosynthetic autotrophs (chemolithotrophs). When growing in the autotrophic mode, they use sulfide as energy source to assimilate CO_2. Most of them oxidize the sulfide to sulfate regardless of the level of oxygen tension [e.g., *Thiobacillus*

thiooxidans (London and Rittenberg, 1964)]. However, some like *Thiobacillus thioparus* form elemental sulfur (S^0) if the pH of their milieu is initially alkaline and the rH_2* is 12, i.e., if the milieu is partially reduced due to an oxygen tension below saturation. Thus, *T. thioparus* T5, isolated from a microbial mat, produces elemental sulfur in continuous culture in a chemostat under conditions of oxygen limitation. In this case, small amounts of thiosulfate together with even smaller amounts of tetrathionate and polysulfide are also formed (van den Ende and van Gemerden, 1993). In batch culture under oxygen limitiation, *T. thioparus* has been observed to produce initially a slight increase in pH followed by a drop to about 7.5 in 4 days and a rise in rH_2 to about 20 (Sokolova and Karavaiko, 1968). The reaction leading to the formation of elemental sulfur can be summarized as

$$HS^- + 0.5O_2 + H^+ \rightarrow S^0 + H_2O \tag{18.13}$$

Under conditions of high oxygen tension (at or near saturation), *T. thioparus* will oxidize soluble sulfide all the way to sulfate (London and Rittenberg, 1964; Sokolova and Karavaiko, 1968; van den Ende and van Gemerden, 1993):

$$HS^- + 2O_2 \rightarrow {SO_4}^{2-} + H^+ \tag{18.14}$$

Thiovulum sp. is another example of a member of the domain Bacteria that oxidizes sulfide to sulfur under reduced oxygen tension (Wirsen and Jannasch, 1978).

London and Rittenberg (1964) (see also Vishniac and Santer, 1957) suggested that the intermediate steps in the oxidation of sulfide to sulfate were

$$4S^{2-} \rightarrow 2{S_2O_3}^{2-} \rightarrow {S_4O_6}^{2-} \rightarrow {SO_3}^{2-} + {S_3O_6}^{2-} \rightarrow 4{SO_3}^{2-} \rightarrow {SO_4}^{2-} \tag{18.15}$$

However, this reaction sequence does not explain the formation of elemental sulfur at reduced oxygen tension. Unless this occurs by way of a specialized pathway, which seems doubtful, a more attractive model of the pathway that explains both processes, the formation of both S^0 and ${SO_4}^{2-}$, in a unified way is one proposed by Roy and Trudinger (1970) (see also Suzuki, 1999; Suzuki et al., 1994; Yamanaka, 1996):

$$\begin{array}{l} S^{2-} \rightarrow X \rightarrow {SO_3}^{2-} \rightarrow {SO_4}^{2-} \\ \qquad \Updownarrow \\ \qquad S^0 \end{array} \tag{18.16}$$

Here X represents a common intermediate in the oxidation of sulfide and of elemental sulfur to sulfite. Roy and Trudinger visualized X as a derivative of

*$rH_2 = -\log[H_2] = \left(\frac{E_h}{0.029}\right) + 2\text{pH}$, because $E_h = -0.029\log[H_2] + 0.058\log[H^+]$.

glutathione or a membrane-bound thiol. It may also be representative of the "intermediate sulfur" described by Pronk et al. (1990). The scheme of Roy and Trudinger permits integration of a mechanism for elemental sulfur oxidation into a unified pathway for oxidizing reduced forms of sulfur. Hallberg et al. (1996) found this mechanism consistent with the action of *T. caldus* on reduced forms of sulfur.

Sorokin (1970) questioned the sulfide-oxidizing ability of thiobacilli, believing that they oxidize only thiosulfate resulting from chemical oxidation of sulfide by oxygen and that any elemental sulfur formed by thiobacilli from sulfide is due to the chemical interaction of bacterial oxidation products with S^{2-} and $S_2O_3^{2-}$, as previously proposed by Nathansohn (1902) and Vishniac (1952). This view is not accepted today. Indeed, Vainshtein (1977) and others have presented clear evidence to the contrary. Most recently, Nübel et al. (2000) showed that hyperthermophilic, microaerophilic, chemolithotrophic *Aquifex aeolicus* VF5 oxidizes sulfide to elemental sulfur using a membrane-bound electron transport pathway that conveys electrons from sulfide being oxidized to oxygen. The pathway includes a quinone pool and a cytochrome bc complex and cytochrome oxidase.

Anaerobic Attack

Most bacteria that oxidize sulfide anaerobically are photosynthetic autotrophs (Chlorobiaceae, Chromatiaceae, some Rhodospirillaceae, and a few Cyanobacteria), but a few, like the facultative anaerobes *Thiobacillus denitrificans* and *Thermothrix thiopara*, are chemosynthetic autotrophs. In the presence of nonlimiting concentrations of sulfide, most photosynthetic autotrophs oxidize sulfide to elemental sulfur, using the reducing power from this reaction in the assimilation of CO_2. However, some exceptional organisms exist that never form elemental sulfur (see Sec. 18.6). When elemental sulfur is formed, it is usually accumulated intracellularly by purple sulfur bacteria and extracellularly by green sulfur bacteria and cyanobacteria. Elemental sulfur accumulated extracellularly by *Chlorobium* appears to be readily available to the cell that formed it but not to other individuals in a population of the same organism or to other photosynthetic bacteria that can oxidize elemental sulfur. The sulfur is apparently attached to the cell surface (van Gemerden, 1986). Very recent study by environmental scanning electron microscopy suggests that the extracellularly deposited sulfur is associated with spinae on the cell surface (Douglas and Douglas, 2000). Spinae are helically arrayed protein that form a hollow tube protruding from the cell surface (Easterbrook and Coombs, 1976). Details of the biochemistry of sulfide oxidation by the photosynthetic autotrophs remain to be explored.

The chemosynthetic autotroph *Thiobacillus denitrificans* can oxidize sulfide to sulfate anaerobically with nitrate as terminal electron acceptor. As

the sulfide is oxidized, nitrate is reduced via nitrite to nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2) (Baalsrud and Baalsrud, 1954; Milhaud et al., 1958; Peeters and Aleem, 1970; Adams et al., 1971; Aminuddin and Nicholas, 1973). Acetylene has been found to cause accumulation of sulfur rather than sulfate in a gradient culture of a strain of *T. denitrificans* using nitrous oxide as terminal electron acceptor. In the absence of acetylene, the gradient culture, unlike a batch culture, did not even accumulate sulfur transiently. It was suggested that acetylene prevents the transformation of S^0 to SO_3^{2-} in this culture (Daalsgaard and Bak, 1992). Polysulfide ($S_{n-1}SH^-$) but not free sulfur appears to be an intermediate in sulfide oxidation to sulfate by this organism (Aminuddin and Nicholas, 1973). The polysulfide appears to be oxidized to sulfite and thence to sulfate (Aminuddin and Nicholas, 1973, 1974a, 1974b). The reaction sequence is like that proposed in reaction (18.16).

Oil field brine from the Coleville oil field in Sasketchewan, Canada, has yielded two microaerophilic strains of bacteria, one (strain CVO) resembling *Thiomicrospira denitrificans* and the other (strain FWKO B) resembling *Arcobacter*. Both of these strains can oxidize sulfide anaerobically with nitrate as electron acceptor (Gevertz et al., 2000). Each can grow autotrophically, but strain CVO can also use acetate in place of CO_2. CVO produces elemental sulfur or sulfate, depending on the sulfide concentration, while reducing nitrate or nitrite to dinitrogen. Strain FWKO B produces only sulfur and reduces nitrate only to nitrite.

Oxidation of Sulfide by Heterotrophs and Mixotrophs

Hydrogen sulfide oxidation is not limited to autotrophs. Most strains of *Beggiatoa* (Fig. 18.1) grow mixotrophically or heterotrophically on sulfide. In the former instance, the organisms derive energy from oxidation of the H_2S. In the latter instance, they apparently use sulfide oxidation to eliminate metabolically formed hydrogen peroxide in the absence of catalase (Burton and Morita, 1964) (see also Sec. 18.6). *Beggiatoa* deposits sulfur granules resulting from sulfide oxidation in its cells external to the cytoplasmic membrane in invaginated, double-layered membrane pockets (Strohl and Larkin, 1978; see also discussion by Ehrlich, 1999). The sulfur can be further oxidized to sulfate under sulfide limitation (Pringsheim, 1967). One strain of *Beggiatoa* has proven to be autotrophic. It was isolated from the marine environment (Nelson and Jannasch, 1983; see also Jannasch et al., 1989, and earlier section). The heterotrophs *Sphaerotilus natans* (prokaryote, domain Bacteria), *Alternaria*, and yeast (eukaryotes; fungi) have also been reported to oxidize H_2S to elemental sulfur (Skerman et al., 1957a, 1957b). It has not been established whether these organisms derive useful energy from this oxidation.

Elemental Sulfur

Aerobic Attack

Elemental sulfur may be enzymatically oxidized to sulfuric acid by certain members of the Bacteria and the Archaea. The overall reaction may be written

$$S^0 + 1.5O_2 + H_2O \rightarrow H_2SO_4 \tag{18.17}$$

Cell extract of *Thiobacillus thiooxidans*, to which catalytic amounts of glutathione were added, oxidized sulfur to sulfite (Suzuki and Silver, 1966). In the absence of formaldehyde as trapping agent for sulfite, thiosulfate was recovered in the reaction mixture (Suzuki, 1965), but this was an artifact due to chemical reaction of sulfite with residual sulfur (Suzuki and Silver, 1966) [see reaction (18.10)]. Sulfite was also shown to be accumulated when sulfur was oxidized by *T. thiooxidans* in the presence of the inhibitor 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), which has been shown to inhibit sulfite oxidation. The stoichiometry when the availability of sulfur was limited was 1 mol sulfite accumulated per mole each of sulfur and oxygen consumed (Suzuki et al., 1992). A sulfur-oxidizing enzyme in *Thiobacillus thioparus* used glutathione as a cofactor to produce sulfite (Suzuki and Silver, 1966). The enzyme in both organisms contained non-heme iron and was classed as an oxygenase. The mechanism of sulfur oxidation is consistent with the model described by reaction (18.16). The glutathione in this instance forms a polysulfide [compound *X* in reaction (18.16)] with the substrate sulfur, which is then converted to sulfite by introduction of molecular oxygen. This reaction appears not to yield useful energy to the cell. Sulfur oxidation to sulfite that does not involve oxygenase but an oxidase with a potential for energy conservation has also been considered. Some experimental evidence supports such a mechanism (see Pronk et al., 1990).

Anaerobic Oxidation of Elemental Sulfur

Few details are known as yet as to how elemental sulfur is oxidized in anaerobes, especially photosynthetic autotrophs. *Thiobacillus denitrificans* appears to follow the reaction sequence in (18.16) except that oxidized forms of nitrogen substitute for oxygen as terminal electron acceptor.

T. ferrooxidans has the capacity to oxidize elemental sulfur anaerobically using ferric iron as terminal electron acceptor (Brock and Gustafson, 1976; Corbett and Ingledew, 1987). This anaerobic oxidation yields enough energy to support growth at a doubling time of about 24 hr (Pronk et al., 1991, 1992).

Disproportionation of Sulfur

Anaerobic marine enrichment cultures consisting predominantly of slightly curved bacterial rods have been shown to contain chemolithotrophic bacteria that were able to grow on sulfur by disproportionating it into H_2S and SO_4^{2-}, but only in the presence of sulfide scavengers such as FeOOH, $FeCO_3$, or MnO_2 (Thamdrup et al., 1993; see also Janssen et al., 1996). The disproportionation reaction can be summarized as

$$4S^0 + 4H_2O \rightarrow SO_4^{2-} + 3H_2S + 2H^+ \qquad (18.18)$$

Added ferrous iron scavenges the sulfide by forming FeS, whereas added MnO_2 scavenges sulfide in a redox reaction in which MnO_2 is reduced to Mn^{2+} by the sulfide, producing SO_4^{2-}, with S^0 a probable intermediate. The scavenging action is needed to propel the reaction in the direction of sulfur disproportionation. In the disproportionation reaction, three pairs of electrons from one atom of sulfur are transferred via an as yet undefined electron transport pathway to three other atoms of sulfur, generating H_2S in reaction (18.18). The sulfur atom yielding the electrons is transformed into sulfate. The transfer of the three pairs of electrons is the source of the energy conserved by the organism for growth and reproduction. This sulfur disproportionation reaction is similar to one that has been observed under laboratory conditions with the photolithotrophic green sulfur bacteria *Chlorobium limicola* subspecies *thiosulfaticum* and *Chl. vibrioforme* under an inert atmosphere in the light in the absence of CO_2. To keep the reaction going, the H_2S produced had to be removed by continuous flushing with nitrogen (see Trüper, 1984b).

A study of sulfur isotope fractionation as a result of sulfur-disproportionation by enrichment cultures from Århus Bay, Denmark, and other sediment sources revealed that the sulfide produced can be depleted in ^{34}S by as much as 7.3–8.6‰ and the corresponding sulfate produced can be enriched in ^{34}S by as much as 12.6–15.3‰. When sulfur disproportionation is coupled to oxidation in nature, it can lead to the formation of sedimentary sulfides that are more depleted in ^{34}S than would be sulfides that are generated through bacterial sulfate reduction alone (Canfield and Thamdrup, 1994).

Sulfite Oxidation

Oxidation by Aerobes

Sulfite may be oxidized by two different mechanisms, one of which includes substrate-level phosphorylation while the other does not, although both yield useful energy through oxidative phosphorylation by the intact cell (see, e.g.,

review by Wood, 1988). In substrate-level phosphorylation, sulfite reacts oxidatively with adenylic acid (AMP) to give adenosine 5′-sulfatophosphate (APS):

$$SO_3^{2-} + AMP \xrightarrow{\text{APS reductase}} APS + 2e \tag{18.19}$$

The sulfate of APS is then exchanged for phosphate:

$$APS + P_i \xrightarrow{\text{ADP sulfurylase}} ADP + SO_4^{2-} \tag{18.20}$$

ADP can then be converted to ATP as follows:

$$2ADP \xrightarrow{\text{adenylate kinase}} ATP + AMP \tag{18.21}$$

Hence the oxidation of 1 mol of sulfite yields 0.5 mol of ATP formed by substrate-level phosphorylation. However, most energy as ATP is gained from shuttling electrons in reaction (18.19) through the membrane-bound electron transport system to oxygen (Davis and Johnson, 1967).

A number of thiobacilli appear to use an AMP-independent sulfite oxidase system (Roy and Trudinger, 1970, p. 214). These systems do not all seem to be alike. The AMP-independent sulfite oxidase of autotrophically grown *Thiobacillus novellus* may use the following electron transport pathway (Charles and Suzuki, 1966):

$$SO_3^{2-} \rightarrow \text{cytochrome c} \rightarrow \text{cytochrome oxidase} \rightarrow O_2 \tag{18.22}$$

The sulfite oxidase of *T. neapolitanus* can be pictured as a single enzyme complex that may react either with sulfite and AMP in an oxidation that gives rise to APS and sulfate or with sulfite and water followed by oxidation to sulfate (Roy and Trudinger, 1970). The enzyme complex then transfers the reducing power that is generated to oxygen. Sulfite-oxidizing enzymes that do not require the presence of AMP have also been detected in *T. thiooxidans*, *T. denitrificans*, and *T. thioparus*. *T. concretivorus* (now considered a strain of *T. thiooxidans*) was reported to shuttle electrons from SO_3^{2-} oxidation via the following pathway to oxygen (Moriarty and Nicholas, 1970):

$$SO_3^{2-} \rightarrow (\text{flavin?}) \rightarrow \text{coenzyme } Q_8 \rightarrow \text{cytochrome b} \rightarrow \text{cytochrome c} \rightarrow \text{cytochrome } a_1 \rightarrow O_2 \tag{18.23}$$

The archaeon *Acidianus ambivalens* appears to possess both an ADP-dependent and an ADP-independent pathway. The former occurs in the cytosol, whereas the latter is membrane associated (Zimmermann et al., 1999).

Oxidation by Anaerobes

Thiobacillus denitrificans is able to form APS reductase (Bowen et al., 1966) that is not membrane-bound, as well as a membrane-bound AMP-independent sulfite

oxidase (Aminuddin and Nicholas, 1973, 1974a, 1974b). Both enzyme systems appear to be active in anaerobically grown cells (Aminuddin, 1980). The electron transport pathway under anaerobic conditions terminates in cytochrome d, whereas under aerobic conditions it terminates in cytochromes aa_3 and cytochrome d. Nitrate but not nitrite acts as electron acceptor anaerobically when sulfite is the electron donor (Aminuddin and Nicholas, 1974b).

Thiosulfate Oxidation

Most chemosynthetic autotrophic bacteria that can oxidize sulfur can also oxidize thiosulfate to sulfate. The photosynthetic, autotrophic, purple and green sulfur bacteria and some purple nonsulfur bacteria oxidize thiosulfate to sulfate as a source of reducing power for CO_2 assimilation (e.g., Trüper, 1978; Neutzling et al., 1985). However, the mechanism of thiosulfate oxidation is probably not the same in all these organisms. The chemosynthetic, aerobic autotrophic *Thiobacillus thioparus* will transiently accumulate elemental sulfur outside its cells when growing in an excess of thiosulfate in batch culture but only sulfate when growing in limited amounts of thiosulfate. *T. denitrificans* will do the same anaerobically with nitrate as terminal electron acceptor (Schedel and Trüper, 1980). The photosynthetic purple bacteria may also accumulate sulfur transiently, but some green sulfur bacteria (Chlorobiaceae) do not (see discussion by Trüper, 1978). Several of the purple nonsulfur bacteria (Rhodospirillaceae) when growing photoautotrophically with thiosulfate do not accumulate sulfur in their cells (Neutzling et al., 1985). Some mixotrophic bacteria oxidize thiosulfate only to tetrathionate.

Thiosulfate is a reduced sulfur compound with sulfur in a mixed valence state. Current evidence indicates that the two sulfurs are covalently linked, the outer or sulfane sulfur of $S : SO_3^{2-}$ having a valence of -1 and the inner or sulfone sulfur having a valence of $+5$. An older view was that the sulfane sulfur had a valence of -2 and the sulfone sulfur one of $+6$.

Charles and Suzuki (1966) proposed that in the oxidation of thiosulfate, it was first cleaved according to the reaction

$$S_2O_3^{2-} \rightarrow SO_3^{2-} + S^0 \tag{18.24a}$$

The sulfite was then oxidized to sulfate,

$$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e \tag{18.24b}$$

and the sulfur was oxidized to sulfate via sulfite as previously described:

$$S \rightarrow SO_3^{2-} \rightarrow SO_4^{2-} \tag{18.24c}$$

Alternatively, thiosulfate oxidation may be preceded by a reduction reaction, resulting in the formation of sulfite from the sulfone and sulfide from the sulfane sulfur:

$$S_2O_3^{2-} + 2e \rightarrow S^{2-} - SO_3^{2-} \tag{18.25}$$

These products are then each oxidized to sulfate (Peck, 1962). In the latter case it is conceivable that sulfur could accumulate transiently by the mechanism suggested by reaction (18.16), but sulfur could also result from asymmetric hydrolysis of tetrathionate resulting from direct oxidation of thiosulfate [see Roy and Trudinger (1970) for detailed discussion],

$$2S_2O_3^{2-} + 2H^+ + 0.5O_2 \rightarrow S_4O_6^{2-} + H_2O \tag{18.26}$$

$$S_4O_6^{2-} + OH^- \rightarrow S_2O_3^{2-} + S^0 + HSO_4^- \tag{18.27}$$

The direct oxidation reaction may involve the enzymes thiosulfate oxidase and thiosulfate-cytochrome c reductase, a thiosulfate activating enzyme (Aleem, 1965; Trudinger, 1961). The thiosulfate oxidase may use glutathione as coenzyme [see summary by Roy and Trudinger (1970) and Wood (1988)].

Thiosulfate may also be cleaved by the enzyme rhodanese, which is found in most sulfur-oxidizing bacteria. It can transfer sulfane sulfur to acceptor molecules such as cyanide to form thiocyanate, for instance. This enzyme may also play a role in thiosulfate oxidation. In anaerobically growing *Thiobacillus denitrificans* strain RT, for instance, rhodanese initiates thiosulfate oxidation by causing formation of sulfite from the sulfone sulfur, which is then oxidized to sulfate. The sulfane sulfur accumulates transiently as elemental sulfur outside the cells, and when the sulfone sulfur is depleted, the sulfane sulfur is rapidly oxidized to sulfate (Schedel and Trüper, 1980). In another strain of *T. denitrificans*, however, thiosulfate reductase rather than rhodanese catalyzes the intial step of thiosulfate oxidation, and both the sulfane and sulfone sulfurs are attacked concurrently (Peeters and Aleem, 1970). *Thiobacillus versutus* (formerly *Thiobacillus* A_2) seems to oxidize thiosulfate to sulfate by a unique pathway (Lu and Kelly, 1983) that involves a thiosulfate-oxidizing multienzyme system that has a periplasmic location (Lu, 1986). No free intermediates appear to be formed from either the sulfane or the sulfone sulfur of thiosulfate.

Pronk et al. (1990) summarized the evidence that supports a model in which *T. ferrooxidans, T. thiooxidans*, and *T. acidophilus* oxidize thiosulfate by forming tetrathionate in an initial step:

$$2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2e \tag{18.28}$$

followed by a series of hydrolytic and oxidative steps whereby tetrathionate is transformed into sulfate with transient accumulation of intermediary sulfur from sulfane-monosulfinic acids (polythionates). Thiosulfate dehydrogenase from *T.*

acidophilus, which catalyzes the oxidation of thiosulfate to tetrathionate, has been purified and partially characterized (Meulenberg et al., 1993).

Disproportionation of Thiosulfate

It has been demonstrated experimentally that some bacteria, like *Desulfovibrio sulfodismutans*, can obtain energy anaerobically by disproportionating thiosulfate into sulfate and sulfide (Bak and Cypionka, 1987; Bak and Pfennig, 1987; Jørgensen, 1990a, 1990b):

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (-19.1\,\text{kcal mol}^{-1} \text{ or } -79.8\,\text{kJ mol}^{-1}) \quad (18.29)$$

The energy from this reaction enables them to assimilate carbon from a combination of CO_2 and acetate. Energy conservation by thiosulfate disproportionation seems, however, paradoxical if the oxidation state of the sulfane sulfur is -2 and that of the sulfone sulfur is $+6$, as formerly believed, because no redox reaction would be required to generate a mole of sulfate and sulfide each per mole of thiosulfate. A solution to this paradox is provided by the report of Vairavamurthy et al. (1993), which demonstrated spectroscopically that the charge density of the sulfane sulfur in thiosulfate is really -1 and that of the sulfone sulfur is really $+5$. Based on this finding, the formation of sulfide and sulfate by disproportionation of thiosulfate would indeed require a redox reaction. Another organism that has been shown to be able to disproportionate thiosulfate is *Desulfotomaculum thermobenzoicum* (Jackson and McInerney, 2000). The addition of acetate to the growth medium stimulated thiosulfate disproportionation by this organism. Thiosulfate disproportionation has also been observed with *Desulfocapsa thiozymogenes* (Janssen et al., 1996).

D. sulfodismutans can also generate useful energy from the disproportionation of sulfite and dithionite to sulfide and sulfate (Bak and Pfennig, 1987). The overall reaction for sulfite disproportionation is

$$4SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^- \ (-14.1\,\text{kcal mol}^{-1} \text{ or } -58.9\,\text{kJ mol}^{-1}) \quad (18.30)$$

For dithionite disproportionation, the reaction is

$$4S_2O_4^{2-} + 4H_2O \rightarrow 3HS^- + 5SO_4^{2-} + 5H^+ \quad (-32.1\,\text{kcal mol}^{-1} \text{ or } -134.0\,\text{kJ mol}^{-1}) \quad (18.31)$$

Desulfovibrio sulfodismutans can also grow on lactate, ethanol, propanol, and butanol as energy sources and sulfate as terminal electron acceptor, like typical

sulfate reducers, but growth is slower than by disproportionation of partially reduced sulfur compounds. Bak and Pfennig (1987) suggest that from an evolutionary standpoint, *D. sulfodismutans*-type sulfate reducers could be representative of the progenitors of typical sulfate reducers.

Perry et al. (1993) suggest that *Shewanella putrefaciens* MR-4, which they isolated from the Black Sea, disproportionates thiosulfate to either sulfide plus sulfite or elemental sulfur plus sulfite. They never detected any sulfate among the products in these reactions. These disproportionations are, however, endogonic ($+7.39$ and $3.84\,\text{kcal mol}^{-1}$ or $+30.98$ and $16.10\,\text{kJ mol}^{-1}$ at pH 7, 1 atm, and 25°C, respectively). Perry and coworkers suggest that in *S. putrefaciens* MR-4, these reactions must be coupled to exogonic reactions such as carbon oxidation.

Thiosulfate disproportionation seems to play a significant role in the sulfur cycle of marine sediments (Jørgensen, 1990a). In Kysing Fjord (Denmark) sediment, thiosulfate was identified as a major intermediate product of anaerobic sulfide oxidation that was simultaneously reduced to sulfide, oxidized to sulfate, and disproportionated to sulfide and sulfate. This occurred at a rapid rate as reflected by a small thiosulfate pool. The metabolic fate of thiosulfate in these experiments was determined by adding differentially labeled ^{35}S-thiosulfate and following the consumption of the thiosulfate and the isotopic distribution in sulfide and sulfate formed from the sulfane and sulfone sulfur atoms of the labeled thiosulfate over time in separate experiments. According to Jørgensen (1990a), the disproportionation reaction can explain the observed large difference in $^{34}S/^{32}S$ in sulfate and sulfides in the sediments. These findings were extended to anoxic sulfur transformations in further experiments with Kysing Fjord sediments and in new experiments with sediments from Braband Lake, Århus Bay, and Aggersund by Elsgaard and Jørgensen (1992). They showed a significant contribution made by thiosulfate disproportionation in anaerobic production of sulfate from sulfide. Addition of nitrate stimulated anoxic oxidation of sulfide to sulfate. Addition of iron as lepidochrocite (FeOOH) caused partial oxidation of sulfide with the formation of pyrite and sulfur and precipitation of iron sulfides.

Tetrathionate Oxidation

Although bacterial oxidation of tetrathionate has been reported, the mechanism of oxidation is still not certain (see Roy and Trudinger, 1970; Kelly, 1982). It may involve disproportionation and hydrolysis reactions. A more detailed scheme was described by Pronk et al. (1990), already mentioned above in connection with thiosulfate oxidation.

A Common Mechanism for Oxidizing Reduced Inorganic Sulfur Compounds in the Domain Bacteria

Friedrich et al. (2001) suggest that the mechanisms for oxidizing inorganic reduced sulfur compounds by aerobic and anaerobic sulfur-oxidizing bacteria, including anoxygenic phototrophic bacteria, have certain common features. Their suggestion is based on molecular comparisons of the Sox genes and the proteins they encode between those in *Paracoccus pantotrophus* and those in other bacteria capable of oxidizing inorganic reduced sulfur compounds. The Sox enzyme system in the archeon *Sulfolobus sulfataricus* appears to differ from that in Bacteria on the basis of genomic analysis.

18.8 AUTOTROPHIC AND MIXOTROPHIC GROWTH ON REDUCED FORMS OF SULFUR

Energy Coupling in Bacterial Sulfur Oxidation

All evidence to date indicates that to conserve biochemically useful energy, chemosynthetic autotrophic and mixotrophic bacteria that oxidize reduced forms of sulfur feed the reducing power (electrons) into a membrane-bound electron transport system whether oxygen, nitrate, or nitrite is the terminal electron acceptor (Peeters and Aleem, 1970; Moriarty and Nicholas, 1970; Sadler and Johnson, 1972; Aminuddin and Nicholas, 1974b; Loya et al., 1982; Lu and Kelly, 1983; Smith and Strohl, 1991; Kelly et al., 1993; also see review by Kelly, 1982). The components of the electron transport system, i.e., cytochromes, quinones, and non-heme iron proteins, are not identical in all organisms, however. Whatever the electron transport chain makeup, it is the oxidation state of a particular sulfur compound being oxidized, or more exactly the midpoint potential of its redox couple at physiological pH, that determines the entry point into the electron transport chain of the electrons removed during the oxidation of the sulfur compound. Thus, the electrons from elemental sulfur are generally thought to enter the transport chain at the level of a cytrochrome bc_1 complex or equivalent. Actually, as pointed out earlier, the first step in the oxidation of sulfur to sulfate can be the formation of sulfite by an oxygenation involving direct interaction with oxygen without involvement of the cytochrome system. Only in the subsequent oxidation of sulfite to sulfate is the electron transport system directly involved starting at the level of the cytochrome bc_1 complex or equivalent. Also, as discussed earlier, sulfite may be oxidized by an AMP-dependent or -independent pathway. In either case, electrons are passed into the electron transport system at the level of a cytochrome bc_1 complex or equivalent. In the AMP-dependent pathway, most of the energy coupling can be assumed to be chemiosmotic, i.e., on

average 1 or 2 mol of ATP can be formed per electron pair passed to oxygen by the electron transport system, but in addition 0.5 mol of ATP can be formed via substrate phosphorylation [reactions (18.18)–(18.20)]. By contrast, only 1 or 2 mol of ATP can be formed on average per electron pair passed to oxygen by the AMP-independent pathway.

Chemiosmosis is best explained if it is assumed that the sulfite oxidation half-reaction occurs at the exterior of the plasma membrane (in the periplasm):

$$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2H^+ + 2e \tag{18.32}$$

and the oxygen reduction half-reaction on the inner surface of the plasma membrane (cytoplasmic side):

$$0.5O_2 + 2H^+ + 2e \rightarrow H_2O \tag{18.33}$$

In *Thiobacillus versutus*, a thiosulfate-oxidizing, multienzyme system has been located in the periplasm (Lu, 1986).

The pH gradient resulting from sulfite oxidation and any proton pumping associated with electron transport together with any electrochemical gradient provide the proton motive force for ATP generation by F_0F_1 ATPase. Proton translocation during thiosulfate oxidation has been observed in *Thiobacillus versutus* (Lu and Kelly, 1988). Involvement of energy coupling via chemiosmosis is also indicated for *Thiobacillus neapolitanus* using thiosulfate as energy source. The evidence for this is (1) inhibition of CO_2 uptake by the uncouplers carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and (2) an increase in transmembrane electrochemical potential and CO_2 uptake in response to nigericin (Holthuijzen et al., 1987).

Reduced Forms of Sulfur as Sources of Reducing Power for CO_2 Fixation by Autotrophs

Chemosynthetic Autotrophs

Reduced sulfur is not only an energy source but also a source of reducing power for chemosynthetic autotrophs that oxidize it. Because the midpoint potential for pyridine nucleotides is very reduced compared to the midpoint potential for reduced sulfur couples that could serve as potential electron donors, **reverse electron transport** from the electron-donating sulfur substrate to pyridine nucleotide is required (see Chap. 6). Electrons thus travel up the electron transport chain, i.e., against the redox gradient, to NADP with consumption of ATP providing the needed energy. This applies to both aerobes and those anaerobes that use nitrate as terminal electron acceptor (denitrifiers).

Photosynthetic Autotrophs

In purple sulfur and nonsulfur bacteria, reverse electron transport, a light-independent sequence, is used to generate reduced pyridine nucleotide (NADPH) using ATP from photophosphorylation to provide the needed energy. In green sulfur bacteria as well as cyanobacteria, *light-energized electron transport* is used to generate NADPH (Stanier et al., 1986) (see also discussion in Chap. 6).

CO_2 Fixation by Autotrophs

Chemosynthetic Autotrophs

Insofar as studied, thiobacilli (domain Bacteria) generally fix CO_2 by the Calvin–Benson cycle (see Chap. 6), i.e., by means of the ribulose 1,5-bisphosphate carboxylase pathway. In at least some thiobacilli, the enzyme is detected both in the cytosol and in cytoplasmic polyhedral bodies called carboxysomes (Shively et al., 1973). The carboxysomes appear to contain no other enzyme and may represent a means of regulating the level of carboxylase activity in the cytosol (Beudecker et al., 1980, 1981; Holthuijzen et al., 1986a, 1986b). *Sulfolobus* (domain Archaea) assimilates CO_2 via a reverse, i.e., reductive, tricarboxylic acid cycle (see Brock and Madigan, 1988), like green sulfur bacteria (domain Bacteria) (see Chap. 6).

Photosynthetic Autotrophs

Purple sulfur bacteria, purple nonsulfur bacteria, and those cyanobacteria capable of anoxygenic photosynthesis fix CO_2 by the Calvin–Benson cycle, i.e., via the ribulose 1,5-bisphosphate carboxylase pathway, when growing photoautotrophically on reduced sulfur (see Chap. 6). Green sulfur bacteria, on the other hand, use a reverse, i.e., reductive, tricarboxylic acid cycle mechanism (Stanier et al., 1986). However, *Chloroflexus aurantiacus* uses a 3-hydroxypropionate cycle (see discussion in Chap. 6).

Mixotrophy

Free-Living Bacteria

Some sulfur-oxidizing chemosynthetic autotrophs can also grow mixotrophically (e.g., Smith et al., 1980). Among oxidizers of reduced sulfur, *Thiobacillus versutus* (formerly *Thiobacillus* A_2) is a good model for studying autotrophy, mixotrophy, and heterotrophy. It can even grow anaerobically on nitrate (e.g., Wood and Kelly, 1983; Claassen et al., 1987). The organism can use each of these forms of metabolism depending on medium composition (see review by Kelly, 1982). Another more recently studied example is *Thiobacillus acidophilus* growing on tetrathionate (Mason and Kelly, 1988).

Thiobacillus intermedius, which grows poorly as an autotroph in a thiosulfate–mineral salts medium, grows well in this medium if it is supplemented with yeast extract, glucose, glutamate, or other organic additive (London, 1963; London and Rittenberg, 1966). The organic matter seems to repress the CO_2-assimilating mechanism in this organism but not its ability to generate energy from thiosulfate oxidation (London and Rittenberg, 1966). *T. intermedius* also grows well heterotrophically in a medium containing glucose plus yeast extract or glutathione but not in a glucose–mineral salts medium minus thiosulfate (London and Rittenberg, 1966). It needs thiosulfate or organic sulfur compounds because it cannot assimilate sulfate (Smith and Rittenberg, 1974). A nutritionally similar organism is *Thiobacillus organoparus*, an acidophilic, facultatively heterotrophic bacterium. It was first isolated from acid mine water in copper deposits in Alaverdi (former Armenian SSR). It was found to grow autotrophically and mixotrophically with reduced sulfur compounds (Markosyan, 1973).

Thiobacillus perometabolis cannot grow at all autotrophically in thiosulfate–mineral salts medium but requires the addition of yeast extract, casein hydrolysate, or an appropriate single organic compound in order to utilize thiosulfate as an energy source (London and Rittenberg, 1967). Growth on yeast extract or casein hydrolysate is much less luxuriant in the absence of thiosulfate.

Some marine pseudomonads, which are ordinarily considered to grow heterotrophically, have been shown to grow mixotrophically on reduced sulfur compounds (Tuttle et al., 1974). Growth of the cultures on yeast extract was stimulated by the addition of thiosulfate. The bacteria oxidized it to tetrathionate. The growth stimulation by thiosulfate oxidation manifested itself in increased organic carbon assimilation. A number of other heterotrophic bacteria, actinomycetes, and filamentous fungi are also able to oxidize thiosulfate to tetrathionate (Trautwein, 1921; Starkey, 1934; Guittoneau, 1927; Gittoneau and Keilling, 1927), but whether the growth of any of these is enhanced by this oxidation is unknown at this time. Even if it is not, these organisms may play a role in promoting the sulfur cycle in soil (Vishniac and Santer, 1957).

Unusual Consortia

Very unusual consortia involving invertebrates and autotrophic sulfide-oxidizing bacteria have been discovered in submarine hydrothermal vent communities (Jannasch, 1984; Jannasch and Mottl, 1985; Jannasch and Taylor, 1984). Vestimentiferan tube worms (*Riftia pachyptila*), which grow around the submarine vents, especially white smokers, lack a mouth and digestive tract and harbor special organelles in their body cavity called collectively a trophosome. These organelles when viewed in section under a transmission electron microscope are

seen to consist of tightly packed bacteria (Cavanaugh et al., 1981). Metabolic evidence indicates that these are chemosynthetic, autotrophic bacteria (Felbeck, 1981; Felbeck et al., 1981; Rau, 1981; Williams et al., 1988). Some bacteria have been cultured from trophosomes, but whether any of them are the important symbionts of the worm remains to be established (Jannasch and Taylor, 1984). The bacteria in trophosomes appear to be autotrophic sulfur-oxidizing bacteria that share the carbon they fix with the worm. The worm absorbs sulfide, in the form of HS^-, and oxygen from the water through a special organ at its anterior end called an obtracular plume (Jones, 1981; Goffredi et al., 1997) and transmits these via its circulatory system to the trophosome. The blood of the worm contains hemoglobin for reversible binding of oxygen and another special protein for reversible binding of sulfide, the latter protein to prevent reaction of sulfide with the hemoglobin and its consequent destruction (Arp and Childress, 1983; Powell and Somero, 1983). The bound hydrogen sulfide and oxygen are released at the site of the trophosome.

Somewhat less intimate consortia are found between giant clams and mussels (Mollusca) and autotrophic sulfide-oxidizing bacteria around hydrothermal vents. The bacteria in these instances reside not in the gut of the animals but on their gills (see Jannasch and Taylor, 1984, for discussion; also Rau and Hedges, 1979). These looser consortia between autotrophic sulfide-oxidizing bacteria and mollusks appear not to be restricted to hydrothermal vent communities but also occur in shallow water environments rich in hydrogen sulfide (Cavanaugh, 1983).

18.9 ANAEROBIC RESPIRATION USING OXIDIZED FORMS OF SULFUR AS ELECTRON ACCEPTORS

Reduction of Fully or Partially Oxidized Sulfur

Various forms of oxidized sulfur can serve as terminal electron acceptors in the respiration of some bacteria under anaerobic conditions. The sulfur compounds include sulfate, thiosulfate, and sulfur, among others.

Biochemistry of Dissimilatory Sulfate Reduction

A variety of strictly anaerobic bacteria respire using sulfate as terminal electron acceptor. Many are taxonomically quite unrelated and include members of the domains Bacteria and Archaea (see earlier section). Insofar as is now known, the mechanism by which they reduce sulfate follows a very similar, but not necessarily identical, pattern in all. As presently understood, the enzymatic

reduction of sulfate requires an initial activation by ATP to form adenine phosphatosulfate and pyrophosphate:

$$SO_4^{2-} + ATP \xrightarrow{\text{ATP sulfurylase}} APS + PP_i \quad (18.34)$$

In members of the genus *Desulfovibrio* the pyrophosphate (PP_i) is hydrolyzed to inorganic phosphate (P_i), which helps to pull the reaction in the direction of APS:

$$PP_i + H_2O \xrightarrow{\text{pyrophosphatase}} 2P_i \quad (18.35)$$

The energy in the anhydride bond of pyrophosphate is thus not available to *Desulfovibrio*. By contrast, this energy is conserved by members of the genus *Desulfotomaculum*. They do not hydrolyze the pyrophosphate but use it as a substitute for ATP (Liu et al., 1982). This also has the effect of pulling reaction (18.34) in the direction of APS.

Unlike in assimilatory sulfate reduction, APS, once formed, is reduced directly to sulfite and adenylic acid (AMP):

$$APS + 2e \xrightarrow{\text{APS reductase}} SO_3^{2-} + AMP \quad (18.36)$$

The APS reductase, unlike PAPS reductase, does not require NADP as a cofactor but, like PAPS reductase, contains bound FAD and iron (for further discussion see, for instance, Peck, 1993).

The subsequent details in the reduction of sulfite to sulfide are not fully agreed upon. One line of experimental evidence suggests a multistep process involving trithionate and thiosulfate as intermediates (Kobiyashi et al., 1969; modified by Akagi et al., 1974; Drake and Akagi, 1978):

$$3HSO_3^- + 2H^+ + 2e \xrightarrow{\text{bisulfite reductase}} S_3O_6^{2-} + 2H_2O + OH^- \quad (18.37)$$

$$S_3O_6^{2-} + H^+ + 2e \xrightarrow{\text{trithionate reductase}} S_2O_3^{2-} + HSO_3^- \quad (18.38)$$

$$S_2O_3^{2-} + 2H^+ + 2e \xrightarrow{\text{thiosulfate reductase}} HS^- + HSO_3^- \quad (18.39)$$

In most *Desulfovibrio* cultures, the bisulfite reductase seems to be identical to desulfoviridin (Kobayashi et al., 1972; Lee and Peck, 1971). In *D. desulfuricans* strain Essex 6, both a soluble and a membrane-bound desulfoviridin were detected. The membrane-bound activity amounted to 90% of the total. Unlike the soluble activity, the membrane-bound activity could be coupled to hydrogenase and cytochrome c_3. Sulfide was the main product of reduction with this enzyme (Steuber et al., 1994). In *D. desulfuricans* strain Norway 4, which lacks desulfoviridin, desulforubidin appears to be the bisulfite reductase (Lee et al., 1973), and in *Desulfovibrio thermophilus* it has been identified as desulfofuscidin

(Fauque et al., 1990). In *Desulfotomaculum nigrificans*, a carbon monoxide-binding pigment, called P582 by Trudinger (1970), accounts for bisulfite-reducing activity, which, according to Akagi et al. (1974), leads to the formation of trithionate, with thiosulfate and sulfide as endogenous side products. An F_0F_1-ATP synthase involved in energy conservation from sulfate reduction by *Desulfovibrio vulgaris* has been identified (Ozawa et al., 2000).

Inducible sulfite reduction has also been observed with *Clostridium pasteurianum*, a bacterium that is not a dissimilatory sulfate reducer. It can reduce sulfite to sulfide. In the absence of added selenite, whole cells do not release detectable amounts of trithionate or thiosulfate when reducing sulfite, but in the presence of selenite they do. Selenite was found to inhibit thiosulfate reductase but not trithionate reductase in whole cells, but inhibited both in cell extracts (Harrison et al., 1980). A purified sulfite reductase from *C. pasteurianum* produced sulfide from sulfite. It was also able to reduce NH_2OH, SeO_3^{2-}, and NO_2^- but did not reduce trithionate or thiosulfate (Harrison et al., 1984). Several physical and chemical properties of this enzyme differed from those of bisulfite reductases in sulfate reducers. Its role in *C. pasteurianum* may be in detoxification when excess sulfite is present (Harrison et al., 1984). Peck (1993) referred to the enzymes involved in the transformation of bisulfite to sulfide collectively as bisulfite reductase. Distinct sulfite reductase, trithionate reductase, and thiosulfate reductase were also identified by Peck and LeGall (1982). However, at the time they did not visualize a major role for these enzymes in sulfite reduction to sulfate.

Chambers and Trudinger (1975) questioned whether the trithionate pathway of sulfite reduction is the major pathway of *Desulfovibrio* spp. They found that results of experiments with isotopically labeled $^{35}SO_3^{2-}$, $^{35}SSO_3^{2-}$, and $S^{35}SO_3^{2-}$ could not be reconciled with the trithionate pathway but were more consistent with a pathway involving the assimilatory kind of sulfite reductase. Their view was supported by Peck and LeGall (1982). However, Vainshtein et al. (1981), after reinvestigating this problem, concluded that the findings of Chambers and Trudinger (1975) were the result of using a heavy cell concentration (limiting bisulfite concentration) that did not permit transient accumulation of thiosulfate. LeGall and Fauque (as cited by Fauque et al., 1991) concluded in 1988 that a direct pathway from sulfite to sulfide is used by *Desulfovibrio* and a trithionate pathway by *Desulfotomaculum*. It appears that our understanding of the details of sulfite reduction to sulfide by sulfate-reducing bacteria remains incomplete at this time.

Sulfur Isotope Fractionation

Sulfate-reducing bacteria can distinguish between ^{32}S and ^{34}S isotopes of sulfur; i.e., they can bring about isotope fractionation (Harrison and Thode, 1957; Jones

and Starkey, 1957). Both isotopes are stable. The ^{32}S isotope of sulfur is the most abundant (average 95.1%) and the ^{34}S isotope is the next most abundant (average 4.2%). The $^{32}S/^{34}S$ ratio of unfractionated natural sulfur compounds ranges between 21.3 and 23.2. Meteoritic sulfur has a $^{32}S/^{34}S$ ratio of 22.22. Because this ratio appears to be relatively constant from sample to sample, it is often used as a reference standard against which to compare sulfur isotope ratios of other materials that may be either enriched or depleted in ^{34}S. Isotope fractionation by microbes is the result of preferential attack of ^{32}S over ^{34}S as a consequence of which the sulfur in the product of the attack becomes enriched in ^{32}S. Although early work based on observations with *Desulfovibrio desulfuricans* suggested that sulfur isotope fractionation by sulfate reducers proceeded more readily under conditions of slow growth (Tables 18.5 and 18.6) (Jones and Starkey, 1957, 1962), recent results, albeit with a variety of sulfate reducers that did not include *D. desulfuricans*, did not confirm this observation (Detmers et al., 2001). The nature of the electron donor may affect the degree of isotope fractionation (Kemp

TABLE 18.5 Sulfide Production and Fractionation of Stable Isotopes of Sulfur by *Desulfovibrio desulfuricans* Cultivated at 28°C (Rapid Growth)

Sample number	Incubation period (hr)[a]	Sulfide S in PbS (mg)	Sulfate reduced[b] (%)	Number of isotope determinations	$\delta^{34}S$
1	44	996	6.3	4	−5.4
2	8	2168	20.0	4	−4.9
3	4	1931	32.2	2	−3.1
4	5	1448	41.4	2	−3.1
5	4	1394	50.2	2	−5.4
6	3	1248	58.1	2	−5.4
7	9	317	60.1	2	−6.7
8	14	191	61.3	2	−8.9
9	41	103	62.0	2	−9.8
10	68	115	62.7	2	−12.9
11	59.5	387	65.2	2	−7.2
12	25.5	901	70.9	2	−3.1
13	7	615	74.8	2	−0.5
14	6	474	77.8	2	+0.9
15	24	856	83.2	2	+0.5
16	43	106	83.9	2	−4.9

[a] Periods were calculated from the time sulfide first appeared in the culture substrate. This was 60 hr after the medium was inoculated.
[b] The initial sulfate S in the substrate was 3943 ppm.
Source: Adapted from Jones and Starkey (1957), with permission.

TABLE 18.6 Cultivated at Low Temperatures (Slow Growth)

Sample number	Incubation period (hr)[a]	Sulfide S in PbS (mg)	Sulfate reduced (%)	$\delta^{34}S$
17	200	21.2	4.4	−22.1
18	142	65.8	4.5	−25.9
19	120	112.7	8.3	−25.9
20	120	167.8	10.0	−24.2
21	96	174.6	13.1	−24.2
22	120	180.8	16.0	−22.9
23	144	134.5	16.8	−21.6
24	120	102.0	18.4	−19.5

[a]Periods were calculated from the time sulfide first appeared in the culture substrate; this was 18 hr after the medium was inoculated.
Source: Adapted from Jones and Starkey (1957) with permission.

and Thode, 1968), but the temperature range for growth does not (Böttcher et al., 1999).

The degree of isotope fractionation is calculated in terms of $\delta^{34}S$ values expressed in parts per thousand (‰):

$$\partial^{34}S = \frac{^{34}S/^{32}S \text{ sample} - {}^{34}S/^{32}S \text{ meteoritic standard}}{^{34}S/^{32}S \text{ meteoritic standard}} \times 1000 \qquad (18.40)$$

Harrison and Thode (1957) (see also Hoefs, 1997) proposed that S−O bond breakage was the rate-controlling reaction in bacterial sulfate reduction (i.e., reduction of APS to sulfite and AMP) that is responsible for the isotope fractionation phenomenon.

Dissimilatory sulfate reduction is not the only process that may lead to sulfur isotope fractionation. Sulfite reduction by *Desulfovibrio* and *Saccharomyces cerevisiae* (Kaplan and Rittenberg, 1962, p. 81) and by *Clostridium pasteurianum* (Laishley and Krouse, 1978), sulfide release from cysteine by *Proteus vulgaris* (Kaplan and Rittenberg, 1962, 1964), and assimilatory sulfate reduction by *Escherichia coli* and *S. cerevisiae* (Kaplan and Rittenberg, 1962) can also lead to sulfur isotope fractionation.

Sulfur isotope fractionation has also been observed when thiosulfate is reduced by *Desulfovibrio desulfuricans* (Smock et al., 1998). In this instance it was found that the depletion in ^{34}S of the H_2S formed was 10‰ with respect to the total sulfur in thiosulfate. However, the sulfane (outer) and sulfone (inner) sulfur of thiosulfate contributed differently to the overall fractionation, the sulfone sulfur contributing 15.4‰ and the sulfane sulfur 5.0‰. Although S−O bond

breakage of sulfone sulfur is thought to have contributed significantly to the observed fractionation, Smock et al. (1998) suggest that other factors such as thiosulfate uptake, sulfonate activation, intracellular concentrations, or the physiological state of the cells could influence the observed isotope effect.

Isotopic analysis of sulfur minerals in nature has helped in deciding whether biogenesis was involved in their accumulation. Any given deposit must, however, be sampled at a number of locations, because isotope enrichment values (δ^{34}S) generally fall in a narrow range or a wide range. Abiogenic δ^{34}S values generally fall in a narrow range and usually have a positive sign, whereas biogenic values tend to fall in a wide range and have a negative sign.

Reduction of Elemental Sulfur

Elemental sulfur can be used anaerobically as terminal electron acceptor in bacterial respiration or as an electron sink for disposal of excess reducing power. The product of S^0 reduction in either case is sulfide. Polysulfide may be an intermediate in respiration (Schauder and Müller, 1993; Fauque et al., 1991). Some members of both Bacteria and Archaea can respire on sulfur (Schauder and Kröger, 1993; Schicho et al., 1993; Ma et al., 2000; Bonch-Osmolovskaya, 1994). Examples of Bacteria include *Desulfuromonas acetoxidans, Desulfovibrio gigas*, and some other sulfate reducers (Pfennig and Biebl, 1976; Biebl and Pfennig, 1977; Fauque et al., 1991). Examples of Archaea include *Pyrococcus furiosus* (Schicho et al., 1993), *Pyrodictium* (Stetter, 1985), *Pyrobaculum* (Huber et al., 1987), and *Acidianus*.

Organisms that use S^0 reduction as an electron sink include *Thermotoga* spp. in the domain Bacteria and *Thermoproteus, Desulfurococcus*, and *Thermofilum* in the domain Archaea (Jannasch et al., 1988a, 1988b). These organisms are fermenters that dispose in this way of excess H_2 they produce, which would otherwise inhibit their growth (Bonch-Osmolovskaya et al., 1990; Janssen and Morgan, 1992; Bonch-Osmolovskaya, 1994). It is possible that these organisms can salvage some energy in the disposal of H_2 (e.g., Schicho et al., 1993). Some fungi, e.g., *Rhodotorula* and *Trichosporon* (Ehrlich and Fox, 1967), can also reduce sulfur to H_2S with glucose as electron donor. This is probably not a form of respiration.

The energy source for the sulfur-respiring Archaea is sometimes hydrogen and/or methane but more often organic molecules such as glucose and small peptides, whereas that for Bacteria may be simple organic compounds (e.g., ethanol, acetate, propanol) or more complex organics. In the case of *Desulfuromonas acetoxidans* (domain Bacteria), an electron transport pathway including cytochromes appears to be involved (Pfennig and Biebl, 1976). When acetate is used as an energy source, oxidation proceeds anaerobically by way of the tricarboxylic acid cycle (see Chap. 6). The oxaloacetate required for initiation

of the cycle is formed by carboxylation of pyruvate, which arises from carboxylation of acetate (Gebhardt et al., 1985). Energy is gained in the oxidation of isocitrate and 2-ketoglutarate. Membrane preparations were shown to oxidize succinate using sulfur or NAD as electron acceptor by an ATP-dependent reaction. Similar membrane preparations reduced fumarate to succinate with H_2S as electron donor by an ADP-independent reaction. Menaquinone mediated hydrogen transfer. Protonophores and uncouplers of phosphorylation inhibited reduction of sulfur but not of fumarate. The compound 2-*n*-nonyl-4-hydroxyquinoline *N*-oxide inhibited electron transport to sulfur or fumarate. Together these observations support the notion that sulfur reduction in *D. acetoxidans* involves a membrane-bound electron transport system and that ATP is formed chemiosmotically, i.e., by oxidative phosphorylation when growing on acetate (Paulsen et al., 1986).

The hyperthermophilic Archaea *Thermoproteus tenax* and *Pyrobaculum islandicum*, growing on sulfur and glucose or casamino acids in the case of the former and peptone in the case of the latter, mineralized their carbon substrates completely. They produced CO_2 and H_2S in a ratio of 1 : 2 using the tricarboxylic acid cycle (Selig and Schönheit, 1994).

Reduction of Thiosulfate

Growth and growth yield of some members of the anaerobic and thermophilic and hyperthermophilic Thermotogales were shown to be stimulated in the presence of thiosulfate (Ravot et al., 1995). The test organisms included *Fervidobacterium islandicum, Thermosipho africanus, Thermotoga maritima, Thermotoga neapolitana*, and *Thermotoga* sp. SERB 2665. The last named was isolated from an oil field. All reduced the thiosulfate to sulfide. The Thermotogales in this group are able to ferment glucose among various energy-yielding substrates. The thiosulfate, like sulfur (see, e.g., Janssen and Morgan, 1992), appears to serve as an electron sink by suppressing H_2 accumulation in the fermentation of glucose, for instance. The accumulation of H_2 has an inhibitory effect on the growth of these organisms. The biochemical mechanism by which they reduce thiosulfate remains to be elucidated. *Pyrobaculum islandicum* is able to mineralize peptone by way of the tricarboxylic acid cycle, using thiosulfate as terminal electron acceptor, producing CO_2 and H_2S in a ratio of 1 : 1 (Selig and Schönheit, 1994).

Terminal Electron Acceptors Other Than Sulfate, Sulfite, Thiosulfate, or Sulfur

A few sulfate reducers can grow with Fe(III) (Coleman et al., 1993; Lovley et al., 1993), nitrate, nitrite (McCready et al., 1983; Keith and Herbert, 1983; Seitz and Cypionka, 1986), fumarate (Miller and Wakerley, 1966), or, in the case of

Desulfomonile tiedjei, chloroaromatics (DeWeerd et al., 1990, 1991) as terminal electron acceptors. A few strains of *Desulfovibrio* can even grow on pyruvate or fumarate without an external electron acceptor by generating H_2 as one of the metabolic end products (Postgate, 1952, 1963). *Desulfovibrio gigas* and a few strains of *D. desulfuricans* grow on fumarate by disproportionating it. They reduce a portion of the fumarate to succinate and oxidize the remainder to malate and acetate (Miller and Wakerley, 1966).

When Fe(III) serves as terminal electron acceptor, it may be reduced to $FeCO_3$ (siderite) or Fe^{2+}. When NO_3^- and NO_2^- are the terminal electron acceptors, they are reduced to ammonia (nitrate ammonification). When fumarate is the terminal electron acceptor, it is reduced to succinate. When a chloroaromatic such as 3-chlorobenzoate is the terminal electron acceptor, it is reductively dechlorinated to benzoate and chloride. How many different sulfate-reducing bacteria are able to substitute any of these terminal electron acceptors for sulfate has yet to be systematically explored. The fact that some sulfate reducers can avail themselves of such "substitute" terminal electron acceptors may explain why the presence of such organisms can be detected in environments, like most soils, in which the natural sulfate, sulfite, thiosulfate, or sulfur concentration is very low.

Oxygen Tolerance of Sulfate Reducers

In general, sulfate reducers are strict anaerobes, yet they have shown limited oxygen tolerance (Wall et al., 1990; Abdollahi and Wimpenny, 1990; Marshall et al., 1993). Indeed, *Desulfovibrio desulfuricans, D. vulgaris, D. desulfodismutans, Desulfobacterium autotrophicum, Desulfolobus propionicus*, and *Desulfococcus multivorans* show an ability to use oxygen as terminal electron acceptor, i.e., to respire microaerophilically (below 10 μM dissolved O_2), without, however, being able to grow under these conditions (Dilling and Cypionka, 1990).

Some evidence has been presented in support of aerobic sulfate reduction by bacteria, none of which had been obtained in pure culture until very recently (Canfield and Des Marais, 1991; Jørgensen and Bak, 1991; Fründ and Cohen, 1992). Now, a chemostat study of a coculture of *Desulfovibrio oxyclinae* and *Marinobacter* strain MB, isolated from a mat from Solar Lake in the Sinai Peninsula, showed that *D. oxyclinae* is able to grow slowly on lactate in the presence of air and the concurrent absence of sulfate or thiosulfate. The lactate is oxidized to acetate by *D. oxyclinae* (Krekeler et al., 1997; Sigalevich and Cohen, 2000; Sigalevich et al., 2000a). *Marinobacter* strain MB is a facultatively aerobic heterotroph. When grown on lactate in the presence of sulfate in a chemostat supplied with oxygen after an initial anaerobic growth phase, a pure culture of *D. oxyclinae* tended to form clumps after about 140 hr of exposure to oxygen (Sigalevich et al., 2000b). Such clumps were not formed in coculture with

Marinobacter strain MB (Sigalevich et al., 2000b). The clumping may represent a defense mechanism against exposure to oxygen for sulfate-reducing bacteria in general because the interior of clumps >3 μm in size will become anoxic in the interior.

18.10 AUTOTROPHY, MIXOTROPHY, AND HETEROTROPHY AMONG SULFATE-REDUCING BACTERIA

Autotrophy

Although the ability of *Desulfovibrio desulfuricans* to grow autotrophically with hydrogen (H_2) as energy source had been previously suggested, experiments by Mechalas and Rittenberg (1960) failed to demonstrate it. Seitz and Cypionka (1986), on the other hand, did obtain autotrophic growth of *D. desulfuricans* strain Essex 6 with hydrogen, but the growth yield was small when sulfate was the terminal electron acceptor. Better yields were obtained with nitrate or nitrite as terminal electron acceptor, presumably because the latter two acceptors did not need to be activated by ATP, which is a requirement for sulfate reduction. Nitrate and nitrite are reduced to ammonia by *Desulfovibrio* (McCready et al., 1983; Mitchell et al., 1986; Keith and Herbert, 1983). *Desulfotomaculum orientis* also has the ability to grow autotrophically with hydrogen as energy source and sulfate, thiosulfate, or sulfite as terminal electron acceptor (Cypionka and Pfennig, 1986). Under optimal conditions, better growth yields were obtained with this organism than had been reported for *Desulfovibrio desulfuricans* (12.4 versus 9.7 g of dry cell mass per mole of sulfate reduced). This may be explainable on the basis that *Desulfotomaculum* can utilize inorganic pyrophosphate generated in sulfate activation as an energy source whereas *Desulfovibrio* cannot. *Desulfotomaculum orientis* gave better growth yields when thiosulfate or sulfite was terminal electron acceptor than when sulfate was. The organism excreted acetate that was formed as part of its CO_2 fixation process (Cypionka and Pfennig, 1986). The acetate may have been formed via the activated acetate pathway in which acetate is formed directly from two molecules of CO_2, as is the case in methanogens and homoacetogens (see Chaps. 6 and 21) and as has now been shown in *Desulfovibrio baarsii*, which can also grow with hydrogen and sulfate (Jansen et al., 1984) and in *Desulfobacterium autotrophicum* (Schauder et al., 1989). *Desulfobacter hydrogenophilus*, by contrast, assimilates CO_2 by a reductive tricarboxylic acid cycle when growing autotrophically with H_2 as energy source and sulfate as terminal electron acceptor (Schauder et al., 1987). Other sulfate reducers that are able to grow autotrophically on hydrogen as energy source and sulfate as terminal electron acceptor include *Desulfonema limicola*, *D.*

ishimotoi, and *Desulfosarcina variabilis* (Pfennig et al., 1981; Fukui et al., 1999) and *Desulfobacterium autotrophicum* (Schauder et al., 1989).

Mixotrophy

Desulfovibrio desulfuricans has been shown to grow mixotrophically with any one of several different substances as sole energy source, including hydrogen, formate, and isobutanol. The carbon in the organic energy sources was not assimilated. It was derived instead from substances as complex as yeast extract or as simple as acetate or acetate plus CO_2. Sulfate was the terminal electron acceptor in all instances (Mechalas and Rittenberg, 1960; Sorokin, 1966a, 1966b, 1966c, 1966d; Badziong and Thauer, 1978; Badziong et al., 1978; Brandis and Thauer, 1981). A strain of *D. desulfuricans* used by Sorokin (1966a) was able to derive as much as 50% of its carbon from CO_2 when it grew on hydrogen as energy source and acetate plus CO_2 as carbon source, whereas on lactate plus CO_2 it derived only 30% of its carbon from CO_2. Badziong et al. (1978), using a different strain of *Desulfovibrio*, found that 30% of its carbon was derived from CO_2 when it grew on hydrogen and acetate plus CO_2.

Members of some other genera of sulfate-reducing bacteria can also grow mixotrophically on hydrogen and acetate plus CO_2 (Pfennig et al., 1981). In all these instances, ATP is generated chemiosmotically from hydrogen oxidation in the periplasm.

Heterotrophy

All sulfate reducers can grow heterotrophically with sulfate as terminal electron acceptor. In general, sulfate reducers specialize with respect to the carbon/energy source they can utilize (see Sect. 18.6) (see also Pfennig et al., 1981). When acetate serves as energy source, it may be completely oxidized anaerobically via the tricarboxylic acid cycle, as in the case of *Desulfobacter postgatei* (Brandis-Heep et al., 1983; Gebhardt et al., 1983; Müller et al., 1987). More commonly, however, sulfate reducers oxidize acetate by reversal of the active acetate synthesis pathway (Schauder et al., 1986). Assimilation of acetate most likely involves carboxylation to pyruvate. ATP synthesis in the heterotrophic mode of sulfate reduction, insofar as it is understood, is mainly by oxidative phosphorylation (chemiosmotically) involving transfer of hydrogen abstracted from organic substrates into the periplasm followed by its oxidation (Odom and Peck, 1981; but see also Odom and Wall, 1987; Kramer et al., 1987). In the case of lactate, this hydrogen transfer from the cytoplasm to the periplasm across the plasma membrane appears to be energy-driven (Pankhania et al., 1988). Some ATP may be formed by substrate-level phosphorylation.

18.11 BIODEPOSITION OF NATIVE SULFUR

Types of Deposits

Deposits of elemental sulfur of biogenic origin, and in most cases of abiogenic origin, have resulted from the oxidation of H_2S:

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O \tag{18.41}$$

In some fumaroles, sulfur may also form abiogenically through the interaction of H_2S and SO_2:

$$2H_2S + SO_2 \rightarrow 3S^0 + 2H_2O \tag{18.42}$$

Most known native sulfur deposits are not of volcanogenic origin. Indeed, only 5% of known reserves are the result of volcanism (Ivanov, 1968, p. 139). Biogenic sulfur accumulation in sedimentary deposits may originate syngenetically or epigenetically. In **syngenetic** formation, sulfur is deposited contemporaneously with the enclosing host rock or sediment during its sedimentation. In **epigenetic** formation, sulfur is laid down in cracks and fissures of preformed host rock. This sulfur may originate from a diagenetic process in which a sulfate component of the host rock is converted to sulfur, or it may involve the conversion of dissolved sulfate or sulfide in a solution percolating through cracks and fissures of host rock. Syngenetic sulfur deposits are generally formed in limnetic environments, whereas epigenetic sulfur deposits tend to form in terrestrial environments.

If the source of elemental sulfur is sulfate, the microbial transformation is a two-stage process. The first stage involves dissimilatory sulfate reduction to sulfide (elemental sulfur is not an intermediate in the process), and the second stage involves oxidation of the sulfide to elemental sulfur under limited oxygen availability or anaerobically.

Examples of Syngenetic Sulfur Deposition

Cyrenaican Lakes, Libya, North Africa

A typical example of contemporaneous syngenetic sulfur deposition is found in the sediments of the Cyrenaican lakes Ain ez Zauia, Ain el Rabaiba, and Ain el Braghi. The origin of the sulfur in these lakes was first studied by Butlin and Postgate (1952) and Butlin (1953). The extensive native sulfur in the lake sediments makes up as much as half of the silt. The lake waters have a strong odor of hydrogen sulfide and are opalescent, owing to a fine suspension of sulfur crystals. A fourth lake in the same general area, called Ain amm el Gelud, also contains sulfuretted water but shows no evidence of sulfur in its sediment. Ain ez Zauia was the most thoroughly studied by Butlin and Postgate. It is made up of two adjacent basins, 55×30 m and 90×70 m in expanse, respectively, and no

deeper than 1.5 m. Other characteristics of the lake are summarized in Tables 18.7 and 18.8. The water in the lake is introduced by warm springs (Butlin, 1953). The border of Ain ez Zauia as well as those of the other two lakes with sulfur deposits featured a characteristic red carpetlike gelatinous material that extended several yards into the shallow water in some places. The underside of this red gelatinous material showed a green and black material. Some of the red material was found floating in the water in the form of red bulbous formations. The red material was a massive growth of the photosynthetic purple sulfur bacterium *Chromatium*, and the green material consisted of a growth of the green photosynthetic sulfur bacterium *Chlorobium*. Many sulfate-reducing bacteria were also detected in the lakes. From these qualitative observations Butlin and Postgate (1952) inferred that the sulfate reducers were responsible for producing hydrogen sulfide from the sulfate in the water, using as carbon and energy source some of the organic carbon produced by the photoautotrophic bacteria. Their model can be visualized as a cycle in which the photosynthetic bacteria oxidize the hydrogen sulfide produced by the sulfate-reducing bacteria to elemental sulfur while assimilating CO_2 photosynthetically in the process. The sulfate-reducing bacteria, in turn, use

TABLE 18.7 Physical Characteristics of Lake Ain ez Zauni

Surface area	7950 m^2
Maximum depth	1.5 m
Surface temperature	30°C
Bottom temperature	32°C
Air temperature	16°C
Sulfure production per year	100 tons

Source: Ivanov, 1968.

TABLE 18.8 Chemical Composition of the Waters of Lake Ain ez Zauni

H_2S in surface water: 15–20 mg L^{-1}			
H_2S in bottom water: 108 mg L^{-1}			
Total solids: 25.25 g L^{-1}			
Ca	1179 mg L^{-1}	Cl	13,520 mg L^{-1}
Mg	336 mg L^{-1}	HCO_3	145 mg L^{-1}
Na	7636 mg L^{-1}	SO_4	1848 mg L^{-1}
K	320 mg L^{-1}	NO_3	3 mg L^{-1}
NH_3	8 mg L^{-1}	SiO_3	70 mg L^{-1}

Source: Ivanov, 1968.

some of the fixed carbon produced by the photosynthetic bacteria to reduce sulfate in the lake to sulfide.

Butlin and Postgate recognized that some of the hydrogen sulfide in the lake water could undergo autoxidation to form sulfur, but they considered this process unimportant because the lake Aim amm el Gelud, which contains sulfuretted water, contains an insignificant amount of sulfur in its sediment and also lacks noticeable growth of photosynthetic bacteria. Butlin and Postgate were able to reconstruct an artificial system in the laboratory with pure and mixed cultures of sulfate-reducing and photosynthetic sulfur bacteria that reproduced the process they postulated for sulfur deposition in the Cyrenaican lakes. Significantly, however, they found it best to supplement their artificial lake water with 0.1% sodium malate to achieve good sulfur production. This led to questions about the correctness of their model for biogenesis of sulfur in these lakes.

Ivanov (1968) pointed out that Butlin and Postgate's model did not account for all the carbon needed for sulfur production from sulfate in the Cyrenaican lakes. He argued that a cyclical mechanism in which the photosynthetic sulfur bacteria produce the organic carbon with which the sulfate-reducing bacteria reduce sulfate to sulfide and that the photosynthetic sulfur bacteria then turn into S^0 suffers from carbon limitation. He showed that each turn of a cycle produces only one-fourth or less of the hydrogen sulfide that was produced in the just preceding cycle. As a result, the photosynthetic sulfur bacteria produce only one-fourth or less carbon in each succeeding cycle. This is best illustrated by the reactions

$$2CO_2 + 4H_2S \rightarrow 2(CH_2O) + 4S^0 + 2H_2O \tag{18.43}$$

$$2(CH_2O) + {SO_4}^{2-} + 2H^+ \rightarrow H_2S + 2H_2O + 2CO_2 \tag{18.44}$$

Reaction (18.43) illustrates the photosynthetic reaction, and reaction (18.44) illustrates sulfate reduction. It is seen that to produce the organic carbon (CH_2O) needed for reduce sulfate [reaction (18.43)], four times as much H_2S is consumed as is produced in sulfate reduction [reaction (18.44)]. Ivanov (1968) therefore argued that most of the sulfide turned into sulfur by the photosynthetic sulfur bacteria is introduced into the lake by the warm springs and does not result from sulfate reduction. He noted that Butlin and Postgate (Butlin, 1953) had actually demonstrated that many artesian wells in the area contained sulfuretted water with sulfate-reducing bacteria. Ivanov, however, did not consider the possibility that these wells might also inject H_2 into the lakes that sulfate reducers could employ either autotrophically or mixotrophically as an alternative energy source and reductant of sulfate in carbon-sparing action.

Ivanov (1968) also suggested that a portion of the sulfur in the lake may be produced by nonphotosynthetic sulfur bacteria and by autoxidation. No matter what the source of the H_2S, biogenesis of the sulfur in the Cyrenaican lakes has been confirmed on the basis of stable sulfur isotope analysis (Macnamara and

Thode, 1951; Harrison and Thode, 1958; Kaplan et al., 1960). Figure 18.4 summarizes the different biological reactions by which sulfur may be generated in these lakes.

Lake Sernoye

Lake Sernoye is located in the Kuibishev Oblast in the central Volga region of Russia. It is an artificial, relatively shallow reservoir fed by the Sergievsk sulfuretted springs (Ivanov, 1968). The water output of these springs is around 6,000 m^3 day^{-1}. The waters contain 83–86 mg of H_2S per liter and have a pH of 6.7. The water temperature in summer ranges around 8°C. The lake drains into Molochni Creek. The waters that enter Molochni Creek are reported to be opalescent due to suspended native sulfur in them. The sulfur originates from the oxidation of H_2S in the lake. Much of the lake sediment contains about 0.5% native sulfur, but some sediment contains as much as 2–5%. The lake freezes over in winter, at which time no significant oxidation of H_2S occurs. This fact is reflected in the stratified occurrence of sulfur in the lake sediment. Pure sulfur crystals, which are paragenetic with calcite crystals, have been found in some sediment cores (Sokolova, 1962). Most of the sulfur in the lake is deposited around the sulfuretted springs. At these locations, masses of purple and green sulfur bacteria are seen. Impression smears have shown the presence of *Chromatium* and large numbers of rod-shaped bacteria, which on culturing reveal themselves to be mostly thiobacilli. A study of the H_2S oxidation in Lake Sernoye waters around the springs using $Na_2{}^{35}S$ revealed that the microflora of the lake made a significant contribution (more than 50%). The study differentially measured chemical and biological sulfide oxidation in the dark as well as biological, light-dependent sulfide oxidation. About the same amount of native sulfur was precipitated in the dark and in the light in these experiments, but more sulfate was formed in the light. These results suggested that most of the H_2S in the lake that is biologically oxidized to native sulfur is attacked by thiobacilli, in particular *Thiobacillus thioparus* (Sokolova, 1962). The photosynthetic bacteria appeared to oxidize H_2S for the most part directly to sulfate. They were found to be of the type that is physiologically like *Chromatium thiosulfatophilum*. An average dark production of sulfur during the summer months has been estimated to be 150 kg per day (Ivanov, 1968).

Lake Eyre

Lake Eyre in Australia represents another locality in which evidence of syngenetic sulfur deposition promoted by bacteria has been noted. In shallow water on the southern bank of this lake, sulfur nodules have been found by Bonython (see Ivanov, 1968, pp. 146–150). The nodules are oval to spherical and are usually covered with crusts of crystalline gypsum on the outside while being cavernous

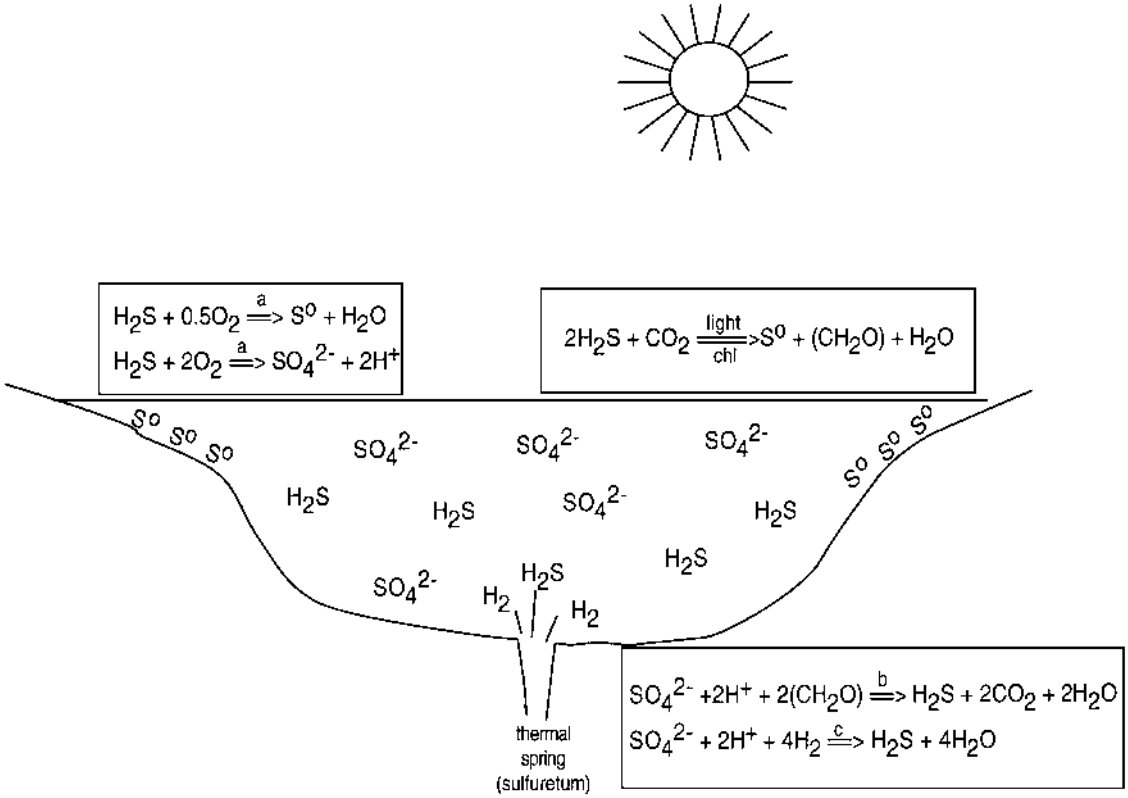


FIG. 18.4 Summary of microbial reactions that can account for the formation of sulfur in the Cyrenaican Lakes, Libya. (a) Caused by thiobacilli in oxidizing zones of the lakes (suggested by Ivanov, 1968) or by autoxidation; (b) caused by sulfate reducers such as *Desulfovibrio desulfuricans*; (c) caused by autotrophic sulfate reducers such as those listed in Table 18.4 [not reported by either Butlin and Postgate (1952) or Ivanov (1968)]. chl = Chlorophyll in green or purple bacteria.

on the inside (Baas Becking and Kaplan, 1956). Their composition includes (in percent by dry weight): $CaSO_4$, 34.8; S^0, 62–63; NaCl, 0.8; Fe_2O_3, 0.45; $CaCO_3$, 0.32; organic carbon, 1.8; and moisture, 7.54 (Baas Becking and Kaplan, 1956). Most nodules as well as the water and muds in the lake were found to contain active sulfate-reducing bacteria and thiobacilli (Baas Becking and Kaplan, 1956). Flagellates and cellulolytic, methane-forming, and other bacteria were also found to abound. Baas Becking and Kaplan (1956) at first proposed that the nodules were forming in the present, with the photosynthetic flagellates providing organic carbon that cellulolytic bacteria convert into a form that can be used by sulfate-reducing bacteria for the reduction of sulfate in gypsum in the surrounding sedimentary rock. The resultant H_2S was then thought to be subjected to chemical and biological (by thiobacilli) oxidation to native sulfur. The nodule structure was seen to result from the original dispersion of the gypsum in septaria in which gypsum is replaced by sulfur. A difficulty with this model, as Ivanov (1968) argues, is that the present oxidation–reduction potential of the ecosystem is +280–340 mV, which is too high for intense sulfate reduction. Sulfate reduction commonly requires a redox potential no higher than around −110 mV.

Radiodating of some nodules has shown them to be 19,600 y old (Baas Becking and Kaplan, 1956). The $^{32}S/^{34}S$ ratios of the sulfur and of the gypsum of the outer crust of the nodules were found to be very similar (22.40–22.56 and 22.31–22.53, respectively), whereas that of the gypsum of the surrounding rock was found to be 22.11. This clearly suggests that the gypsum of the nodule crust is a secondary formation, biogenically produced through the oxidation of the sulfur in the nodules, which was itself biogenically produced in Quaternary time, the age of the surrounding sedimentary deposit. It is possible, therefore, that the sulfur in the nodules derived from H_2S microbiologically generated from the primary gypsum deposit and released into the water and converted to sulfur by organisms like photosynthetic bacteria or *Beggiatoa* residing on the surface of the concretions.

Solar Lake

An example of a lake in which sulfur is produced biogenically but not permanently deposited in the sediment is Solar Lake in the Sinai on the western shore of the Gulf of Aqaba. It is a small hypersaline pond (7000 m^2 surface area, 4–6 m depth), that has undergone very extensive limnological investigations (Cohen et al., 1977a, 1977b, 1977c). It is tropical and has a chemocline (O_2/H_2S interface) and a thermocline that is inverted in winter (i.e., the hypolimnion is warmer than the epilimnion). The chemocline, which is 0–10 cm thick and located at a depth of 2–4 m below the surface, undergoes diurnal migration over a distance of 20–30 cm. The chief cause of this migration is the activity of the cyanobacteria *Oscillatoria limnetica* and *Microcoleus* sp., whose

growth extends from the epilimnion into the hypolimnion. Sulfate-reducing bacteria, including a *Desulfotomaculum acetoxidans* type, near the bottom in the anoxic hypolimnion generate H_2S from the SO_4^{2-} in the lake water. Some of this H_2S migrates upward to the chemocline. During the early daylight hours, H_2S in the chemocline and below is oxidized to elemental sulfur by anaerobic photosynthesis of the cyanobacteria. After they have depleted the H_2S available to them, the cyanobacteria switch to aerobic photosynthesis, generating O_2. Thus, during daylight hours the chemocline gradually drops. After dark, when all photosynthesis by the cyanobacteria has ceased, H_2S generated by the sulfate reducers builds up in the chemocline, together with H_2S generated by the cyanobacteria when they reduce the S^0 they formed earlier with polyglucose they stored from oxygenic photosynthesis. Some of the S^0 is also reduced by bacteria such as *Desulfuromonas acetoxidans*. Thus, during dark hours, the chemocline rises. The cycle is repeated with break of day. Some thiosulfate is found in the chemocline during daylight hours, formed primarily by chemical oxidation of sulfide. This thiosulfate is reduced in the night hours by biological and chemical means. Sulfur thus undergoes a cyclical transformation in the lake such that elemental sulfur does not accumulate to a significant extent. The major driving force of the sulfur cycle is sunlight. [See Jørgensen et al. (1979a, 1979b) for further details of the sulfur cycle in this lake.]

A recent study of cyanobacterial mats in Solar Lake revealed the presence of sulfate reducers in the upper 4 mm, which represents the oxygen chemocline. Significant representation of members of the *Desulfovibrionaceae*, *Desulfobacteriaceae*, and *Desulfonema*-like bacteria was observed by using appropriate rRNA probes (Minz et al., 1999a, 1999b).

Thermal Lakes and Springs

An example of syngenetic sulfur deposition in a thermal lake in which bacteria appear to play a role is found in Lake Ixpaca, Guatemala. This body of water is a crater lake that is supplied with H_2S from **solfataras** (fumarolic hot springs that yield sulfuretted waters), which discharge water at a temperature of 87–95°C (Ljunggren, 1960). The water in the lake has a temperature in the range of 29–32°C. The H_2S concentration of the lake water was reported to be 0.10–0.18 $g\,L^{-1}$. Some of it is oxidized to native sulfur, rendering the water of the lake opalescent. A portion of this sulfur settles out and is incorporated into the sediment. Another smaller portion of the sulfur is oxidized to sulfuric acid, acidifying the lake to a pH of 2.27. The sulfate content of the lake water was reported in the range from 0.46–1.17 $g\,L^{-1}$. The sulfuric acid in the lake water is very corrosive. It decomposes igneous minerals such as pyroxenes and feldspars into clay minerals (e.g., pickingerite). Ljunggren (1960) found an extensive

presence of *Beggiatoa* in the waters associated with the sediments of this lake. He implicated this microorganism in the conversion of H_2S into native sulfur. He suggested that microorganisms also may have a role in the formation of the sulfuric acid, as they do in some hot springs in Yellowstone National Park in the United States (Brock, 1978; Ehrlich and Schoen, 1967; Schoen and Ehrlich, 1968; Schoen and Rye, 1970). More recent studies of solfataras elsewhere led to the discovery of a number of thermophiles, mostly in the domain Archaea such as *Acidianus* (Brierley and Brierley, 1973; Segerer, et al., 1986), *Sulfolobus* (Brock et al., 1972), *Thermoproteus* (Zillig et al., 1981), *Thermofilum pendens* (Zillig et al., 1983), *Sulfurococcus yellowstonii* (Karavaiko et al., 1994), and *Desulfurococcus* (Zillig et al., 1982), but also occasional members of the Bacteria, such as *Thermothrix thiopara* (Caldwell et al., 1976), which have a capacity either to oxidize H_2S, S^0, or thiosulfate or reduce S^0.

Studies of some hot springs in Yellowstone National Park (Brock, 1978) showed that in most the H_2S emitted in their discharge appears to be chemically oxidized to sulfur. A major exception is Mammoth Hot Springs in which H_2S is biochemically oxidized to sulfur as deduced from sulfur isotope fractionation studies. Physiological evidence for bacterial H_2S oxidation was also obtained at Boulder Springs in the park. Unlike most of the springs, its water has a pH in the range of 8–9. Here oxidation can occur at a temperature as high as 93°C (80–90°C, optimum). The bacteria are mixotrophic in this case, being able to use H_2S or other reduced sulfur compounds as energy source and organic matter as carbon source (Brock et al., 1971). Further study of the oxidation of elemental sulfur to sulfuric acid in the acid hot springs in the park revealed that *Thiobacillus thiooxidans* was responsible at temperatures below 55°C and *Sulfolobus acidocaldarius* at temperatures between 55 and 85°C (Fliermans and Brock, 1972; Mosser et al., 1973). Almost all sulfur oxidation in the hot acid springs and hot acid soils was biochemical, because sulfur appeared to be stable in the absence of bacterial activity (Mosser et al., 1973).

Sulfolobus acidocaldarius consists of spherical cells that frequently form lobes and lack peptidoglycan in their cell wall (Brierley, 1966; Brock et al., 1972) (Fig. 15.5). The organism is acidophilic (optimum pH 2–3; pH range 0.9–5.8) and thermophilic (temperature optimum 70–75°C; range 55–80°C). It has a guanine/cytosine ratio of 60–69 mol%. In growing cultures in the laboratory, the growth rate parallels the oxidation rate of elemental sulfur crystals. The presence of yeast extract in the medium was found to partially inhibit sulfur oxidation but not growth. The growth rates of *S. acidocaldarius* in several hot springs in Yellowstone National Park exhibit steady-state doubling times on the order of 10–20 hr in the water of small springs having volumes of 20–2,000 L, and on the order of 30 days in large springs with 1×10^6 L volumes. Doubling times during exponential growth in the water of artificially drained springs were on the order of a few hours (Mosser et al., 1974).

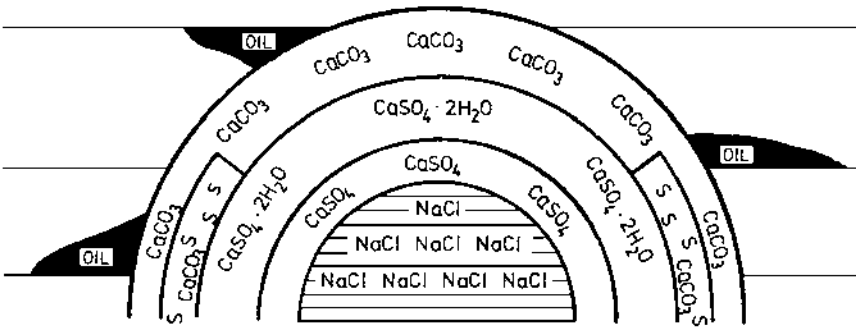


FIG. 18.5 Diagrammatic representation of a salt dome. (After Ivanov, 1968.)

In effluent channels from alkaline hot springs in Yellowstone National Park where the temperature does not exceed 70°C, a bacterium called *Chloroflexus aurantiacus* was discovered (Brock, 1978; Pierson and Castenholz, 1974). It is characterized as a gliding, filamentous (0.5–0.7 μm in width, variable in length), phototrophic bacterium with a tendency to form orange mats below, and to a lesser extent above, thin layers of cyanobacteria such as *Synechococcus* (Doeml and Brock, 1974, 1977). Its photosynthetic pigments include bacteriochlorophylls a and c, and β- and γ-carotene. The pigments occur in *Chlorobium*-type vesicles. Anaerobically, the organism can grow photoautotrophically in the presence of sulfide and bicarbonate (Madigan and Brock, 1975), but it can also grow photoheterotrophically with yeast extract and certain other organic supplements. Aerobically, the organism is capable of heterotrophic growth in the dark. Although showing some physiological resemblance to Rhodospirillaceae (purple nonsulfur bacteria) and even greater resemblance to Chlorobiaceae (green sulfur bacteria), phylogenetically it is related to neither (Brock and Madigan, 1988).

The mats formed by *Chloroflexus* in some hot springs in Yellowstone National Park may be models for the formation of ancient stromatolites. They often incorporate detrital silica in the form of siliceous sinter from the geyser basins and in time are transformed into structures recognizable as stromatolites.

Sulfate in the mats may be reduced to sulfide by sulfate reducers below the upper 3 mm, and the sulfide can be converted to elemental sulfur by *Chloroflexus* in the mats (Doeml and Brock, 1976). This is another example where below 70°C at least some elemental sulfur in hot springs or their effluent may be of biogenic origin.

A study of bacterial mats in hot springs in southwestern Iceland revealed the presence of 14 bacterial types and five archaeal types in mats loaded with precipitated sulfur formed in sulfide-rich water at 60–80°C. Mats formed in low-sulfide springs at 65–70°C were dominated by *Chloroflexus* (Skirnisdottir et al., 2000).

Examples of Epigenetic Sulfur Deposits

Sicilian Sulfur Deposits

An example of epigenetic sulfur deposition in which microbes must have played a role is found on the volcanic Mediterranean island of Sicily. Isotopic studies (Jensen, 1968) showed that the sulfur in these deposits is significantly enriched in ^{32}S relative to the associated sulfate. This finding signifies that the sulfur could not have originated from volcanic activity but must have been the result of microbial sulfate reduction in evaporite deposits that originated from the Mediterranean Sea. The biological agents must have been a consortium of dissimilatory sulfate-reducing bacteria that reduced the sulfate in the evaporite to sulfide and chemosynthetic sulfide-oxidizing bacteria that formed sulfur from the sulfide. Abiotic sulfide oxidation could also have contributed to sulfur formation. Organic carbon, if used in the microbial reduction of the sulfate, came presumably from organic detritus in the sediment (algal or other remains).

Salt Domes

Another example of biogenic native sulfur of epigenetic origin in a sedimentary environment is that associated with salt domes (Fig. 18.5) such as are found on the Gulf Coast of the United States and Mexico (northern and western shores of the Gulf Coast, including those of Texas, Louisiana, and Mexico) (see, e.g., Ivanov, 1968, pp. 92ff; Martinez, 1991). Such salt domes reside directly over a central plug consisting of 90–95% rock salt (NaCl), 5–10% anhydrite ($CaSO_4$), and traces of dolomite ($CaMgCO_3$), barite ($BaSO_4$), and celestite ($SrSO_4$). Petroleum may be entrapped in peripheral deformations. The domes consist mainly of anhydrite topped by calcite, which may have exploitable petroleum associated with it. Between the calcite and anhydrite exists a zone containing gypsum ($CaSO_4 \cdot 2H_2O$), calcite and anhydrite relicts. Sulfur is associated with the calcite in this intermediate zone. The salt domes originated from evaporite, which formed in a period between the late Paleozoic (230–280 million years ago) and the Jurassic (Middle Mesozoic, 135–180 million years ago). A current theory (see Strahler, 1977) proposes that the salt domes on the Gulf Coast began as beds

of evaporite along the continental margins of the newly emergent Atlantic Ocean about 180 million years ago. The evaporite derived from hypersaline waters with the aid of heat emanating from underlying magma reservoirs in these tectonically active areas. As the ocean basin broadened due to continental drift, and as the continental margins became more defined, turbidity currents began to bury the evaporite beds under ever thicker layers of sediment. Ultimately, these sediment layers became so heavy that they forced portions of the evaporite, which has plastic properties, upward as fingerlike salt plugs through ever younger sediment strata. As these plugs intruded into the groundwater zone, they lost their more water-soluble constituents, particularly the rock salt, leaving behind relatively insoluble constituents, especially anhydrite, which became the cap rock. In time, some of the anhydrite was converted to more soluble gypsum, and some was dissolved away. At that point, bacterial sulfate reduction is thought to have begun, lasting perhaps for a period of 1 million years. The active bacteria were most likely introduced into the dome structures from the native flora in the groundwater. The organic carbon needed for bacterial sulfate reduction is thought to have derived from adjacent petroleum deposits.

When the biological contribution to sulfur formation in the salt domes was first recognized (Jones et al., 1956), the only known sulfate reducer was *Desulfovibrio desulfuricans*, which uses lactate, pyruvate, and in most instances malate as energy sources, which it oxidizes to acetate with sulfate as electron acceptor. For *D. desulfuricans* to have used petroleum constituents as a carbon source, it would have had to depend on other bacteria that could transform the constituents, preferably anaerobically, into carbon substrates it could metabolize.

A more recent observation indicated the existence of a sulfate reducer that can use methane (CH_4) as a source of energy (Panganiban and Hanson, 1976; Panganiban et al., 1979). Methane is a major gaseous constituent associated with petroleum deposits.

Very recent studies revealed the existence of sulfate reducers with an ability to use some short-chain saturated aliphatic (including chain lengths of C_8–C_{16}) or aromatic hydrocarbons or heterocyclic compounds, many of which they mineralize (see Sect. 18.6) (see also Aeckersberg et al., 1991; Rueter et al., 1994). Many sulfate reducers have also been shown to be able to use H_2 as their energy source. H_2 occurs in detectable amounts in oil wells. Thus it is not necessary to postulate the past existence of a complex assemblage of anaerobic, fermentative bacteria that converted petroleum hydrocarbons into energy sources for sulfate-reducing bacteria in salt domes.

Whichever sulfate-reducing bacteria were active in the salt domes, they produced not only H_2S but also CO_2. The CO_2 arose from fermentation and/or mineralization of the organic carbon consumed for energy conservation in sulfate reduction. The H_2S was subsequently oxidized biologically and/or chemically to native sulfur, whereas the CO_2 was extensively precipitated as carbonate

(secondary calcite) with the calcium from the anhydrite or gypsum attacked by the sulfate-reducing bacteria.

$$CaSO_4 + 2(CH_2O) \rightarrow CaS + 2CO_2 + 2H_2O \quad (18.45)$$

$$CaS + CO_2 + H_2O \rightarrow CaCO_3 + H_2S \quad (18.46)$$

$$H_2S + 0.5O_2 \rightarrow S^0 + H_2O \quad (18.47)$$

Mineralogical and isotopic study has shown that the sulfur and secondary calcite are physically associated in the cap rock (paragenetic). The isotopic enrichment of the sulfur and secondary calcite indicate a biological origin (Jones et al., 1956; Thode et al., 1954). The sulfur exhibits enrichment with respect to ^{32}S, and the secondary calcite with respect to ^{12}C (see discussion by Ivanov, 1968). Although the enrichment of the secondary calcite in ^{12}C was originally attributed to degradation of petroleum hydrocarbons by sulfate reducers, it seems more likely that it may have been due, at least in part if not entirely, to anaerobic oxidation of ^{12}C-enriched biogenic methane by sulfate reducers, as apparently happened in a native sulfur deposit at Machéw, Poland (Böttcher and Parafiniuk, 1998). A recent study by Detmers et al. (2001) showed that sulfur isotope fractionation was greater when the sulfate reducers mineralized the carbon they consumed than when they oxidized it incompletely. The degree of sulfur isotope fractionation appears to be affected by the metabolic pathway and by regulation of transmembrane sulfate transport (Detmers et al., 2001).

Gaurdak Sulfur Deposit

Epigenetic sulfur deposition in a mode somewhat similar to that associated with the salt domes in the United States took place in the Gaurdak Deposit in Turkmenistan (Ivanov, 1968). This deposit resides in rock of Upper Jurassic age and was probably emplaced in the Quaternary as plutonic waters picked up organic carbon from the Kugitang Suite containing bituminous limestone and sulfate from the anhydrite–carbonate rocks of the Gaurdak Suite. Sulfate-reducing bacteria that entered the plutonic waters reduced the sulfate in it to H_2S with the help of reduced carbon derived from the bituminous material. *Thiobacillus thioparus* oxidized the H_2S to S^0 at the interface where the plutonic water encountered infiltrating oxygenated surface water. Where the sulfur presently encounters oxygenated water, intense bio-oxidation of sulfur to sulfuric acid has been noted, causing transformation of secondary calcite, formed during sulfate reduction in the initial phase of sulfur genesis, into secondary gypsum. The bacteria *T. thioparus* and *T. thiooxidans* have been found in significant numbers in sulfuretted waters in the sulfur deposits with paragenetic (secondary) calcite and in acidic sulfur deposits with secondary gypsum, respectively. Sulfur appears, therefore, to be deposited and degraded in the Gaurdak formation at the present time.

Shor-Su Sulfur Deposit

Another example of epigenetic microbial sulfur deposition is the Shor-Su Deposit in the northern foothills of the Altai mountain range in the southeast corner of the West Siberian Plain. Here an extensive, folded sedimentary formation of lagoonal origin and mainly of Paleocene and Cretaceous age contains major sulfuretted regions in lower Paleocene strata (Bukhara and Suzak) of the second anticline and to a lesser extent in Quaternary conglomerates (Fig. 18.6) (see Ivanov, 1968, pp. 33–34). The sulfur of the main deposits occurs in heavily broken rock surrounded by gypsified rock. It contains some relict gypsum lenses. It is enclosed in a variety of cavernous rock and associated with calcite and celestite in the Bukhara stratum and in cavities and slitlike caves in the Suzak stratum. Petroleum and natural gas deposits are associated with the second anticline, which contains most of the sulfur. One basis for the claim of hydraulic connection between the two anticlines is that their pore waters are chemically very similar in composition. Sulfate-reducing bacteria occur in the plutonic waters that flow through the permeable strata from the fourth to the second anticline. It is believed that these bacteria have been reducing the sulfate that the plutonic waters picked up from dissolution of some of the gypsum and anhydrite in the surrounding rock. The bacteria are presumed to have been using petroleum hydrocarbons or derivatives from them as

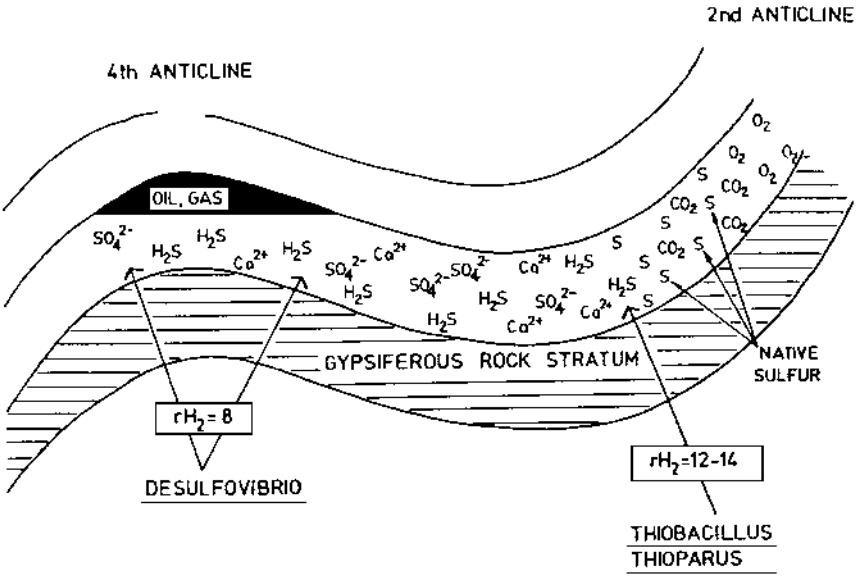


FIG. 18.6 Diagrammatic representation of essential features of the Shor-Su formation. (After Ivanov, 1968.)

a source of energy (reducing power) and carbon for the process. The presence of sulfate-reducing bacteria has been reported in waters of the second anticline and in any rock in which sulfur occurs (Ivanov, 1968). These bacteria were demonstrated to be able to reduce sulfate under in situ conditions at a measurable rate ($0.009–0.179\,mg\ H_2S\,L^{-1}\ day^{-1}$). Native sulfur has been forming where rising plutonic water has been mixing with downward-seeping oxygenated surface water. In this zone of mixing of the two waters, *T. thioparus* was detected and shown to oxidize H_2S from the plutonic water to native sulfur.

Measurements have shown that sulfate reduction predominates where plutonic waters carry sulfate derived from surrounding gypsiferous rock and organic matter derived from associated petroleum. The waters at these sites in the deposit have an rH_2 that often is below 8, indicating strong reducing conditions. The H_2S is transported by the moving plutonic waters to a region in the second anticline, where it encounters aerated surface waters. The waters here have an rH_2 around 12–14 (16.5 maximum). In this environment, *T. thioparus* is favored. It causes conversion of H_2S into S^0. Where the rH_2 exceeds 16.5 owing to extensive exposure to surface water, as in the outcroppings of the western conglomerate of the Shor-Su, the sulfur is undergoing extensive oxidation by *T. thiooxidans*. The pH is found to drop from neutrality to less than 1 where the bacteria are most active. Although unknown then, it is possible that at very low pH hyperacidophilic archaea may be active, as at Iron Mountain, California (Edwards et al., 2000). The sulfur in the main strata began to be laid down in the Quaternary, according to Ivanov (1968). Deposition continues to the present day. For this reason, events in the geological past can be reconstructed from current observations of bacterial distribution and activity in the Quaternary strata of the Shor-Su (Ivanov, 1968).

Kara Kum Sulfur Deposit

Spatially, a somewhat different mechanism of epigenetic sulfur deposition has been recognized in the Kara Kum Deposit north of Ashkhabad in Turkmenistan (Ivanov, 1968). Sulfate-reducing bacteria and H_2S-oxidizing bacteria have also been playing a role in sulfur formation at this site. However, sulfate reduction has been taking place in a different stratum from that involving sulfur deposition, implying that these two activities are spatially separated. The H_2S has been transported to another site before conversion to sulfur. Hence, paragenetic (secondary) calcite is not found associated with the sulfur in this deposit, and consequently sulfuric acid formed at sites of outcropping of the sulfur deposit cannot form gypsum but reacts with sandstone, liberating aluminum and iron, which are precipitated as oxides in a more neutral environment.

18.12 MICROBIAL ROLE IN THE SULFUR CYCLE

As the foregoing discussion shows, microbes play an important role in inorganic as well as organic sulfur transformations (Trüper, 1984a). Figure 18.7 shows how these various biological interactions fit into the sulfur cycle in soil, sediment, and aquatic environments. Although some of these transformations such as aerobic oxidation of H_2S or S^0 may proceed partly by an abiotic route, albeit often significantly more slowly than by a biotic route, at least two other transformations, the anaerobic oxidation of H_2S or S^0 to sulfuric acid and the reduction of sulfate to H_2S, do not proceed readily abiotically at atmospheric pressure in the temperature range that prevails at the Earth's surface. Sulfate reduction is now recognized to be an important mechanism of anaerobic mineralization of organic carbon in anaerobic estuarine and other coastal environments where plentiful sulfate is available from seawater (Skyring, 1987). Geochemically, sulfur-oxidizing and -reducing bacteria are important catalysts in the sulfur cycle in the biosphere.

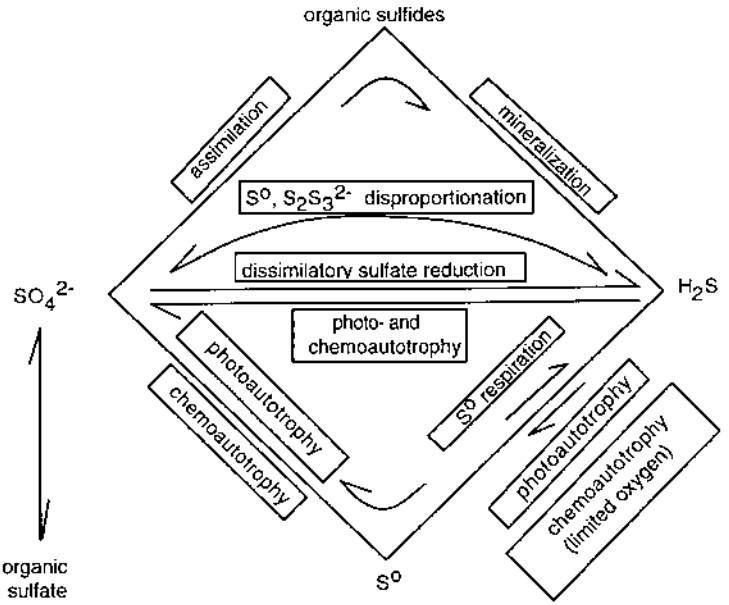


FIG. 18.7 The sulfur cycle.

18.13 SUMMARY

Sulfur, which occurs in organic and inorganic form in nature, is essential to life. Different organisms may assimilate it in organic or inorganic form. Plants and many microbes normally take it up as sulfate. Microbes are important in mineralizing organic sulfur compounds in soil and aqueous environments. The biogeochemistry of organic sulfur mineralization as well as the synthesis of organic sulfur compounds has been studied in some detail.

Inorganic sulfur may exist in various oxidation states in nature, most commonly as sulfide (−2), elemental sulfur (0), and sulfate (+6). Thiosulfate and tetrathionate, each with sulfur in mixed oxidation states, may also occur in significant amounts in some environments. Some microbes in soil and water play an important role in the interconversion of these oxidation states. These include several different members of the domain Bacteria (even certain cyanobacteria under special conditions) and members of the domain Archaea. Among bacteria that oxidize reduced forms of sulfur are chemolithotrophs, anoxygenic and oxygenic (cyanobacterial) photolithotrophs, mixotrophs, and heterotrophs. Most chemolithotrophs and mixotrophs use oxygen as oxidant, but a few chemolithotrophs can substitute nitrate or ferric iron when oxygen is absent. Some chemolithotrophs, such as *T. thioparus*, can oxidize H_2S to S^0 under partially reduced conditions, but they form H_2SO_4 under fully oxidizing conditions. The anoxygenic photolithotrophic bacteria (purple and green bacteria) oxidize H_2S to S^0 or H_2SO_4 to generate reducing power for CO_2 fixation and/or ATP synthesis. Certain cyanobacteria oxidize H_2S to S^0 in the absence of oxygen for generating energy and reducing power for CO_2 fixation. Various chemolithotrophs and mixotrophs can oxidize S^0 to H_2SO_4 aerobically in neutral or acid environments. Sulfur oxidation has been noted in mesophilic and thermophilic environments, in the latter instance at temperatures exceeding 100°C in some cases. Thiosulfate is readily oxidized by some chemolithotrophs, mixotrophs, and heterotrophs. Some marine pseudomonads have been shown to use it as a supplemental energy source, oxidizing it to tetrathionate. Some bacteria can conserve energy by disproportionating elemental sulfur, dithionite, sulfite, or thiosulfate under anaerobic conditions to sulfide and sulfate.

Oxidized forms of sulfur may be reduced by various microorganisms. Elemental sulfur is reduced to H_2S with or without energy conservation by some anaerobic members of the Bacteria and Archaea. Among the Bacteria that conserve energy are *Desulfuromonas acetoxidans, Desulfovibrio gigas*, and some other sulfate-reducing bacteria. Among the Archaea that conserve energy are *Pyrococcus furiosus, Pyrodictium*, and *Acidianus*. Two fungi, *Rhodotorula* and *Trichosporon*, have also been found to be able to reduce S^0 to H_2S, but probably without energy conservation.

Sulfate may be reduced in sulfate respiration (dissimilatory sulfate reduction) by a number of specialized bacteria. Most known species are members of the domain Bacteria, but at least two species are known among the members of the domain Archaea. This microbial activity is of major importance geologically because under natural conditions at the Earth's surface sulfate cannot be reduced by purely chemical means because of the high activation energy required by the process. Sulfate is reduced aerobically by various microbes and plants, but only in small amounts without any extracellular accumulation of H_2S (assimilatory sulfate reduction). The mechanisms of dissimilatory and assimilatory sulfate reduction differ biochemically.

Some reducers and oxidizers of sulfur and its compounds can distinguish between the stable isotopes ^{32}S and ^{34}S and can bring about isotope fractionation. Geologically, this is useful in determining whether ancient sulfur deposits were formed biogenically or abiogenically.

Contemporary biogenic sulfur deposition involving sulfate-reducing bacteria and aerobic and anaerobic sulfide-oxidizing bacteria have been identified in several lacustrine environments. These represent syngenetic deposits. Bacterial oxidation of elemental sulfur to sulfuric acid in certain hot springs has also been reported.

Ancient epigenetic sulfur deposits of microbial origin have been identified in salt domes and other geological formations associated with hydrocarbon (petroleum) deposits in various parts of the world. The sulfur in these instances arose from bacterial reduction of sulfate derived from anhydrite or gypsum followed by bacterial oxidation to elemental sulfur under partially reduced conditions. On full exposure to air, some of the elemental sulfur is presently being oxidized by bacteria to sulfuric acid.

Less spectacular oxidative and reductive transformations of sulfur occur in soil, where they play an important role in the maintenance of soil fertility.

REFERENCES

Abdollahi H, Wimpenny JWT. 1990. Effects of oxygen on the growth of *Desulfovibrio desulfuricans*. J Gen Microbiol 136:1025–1030.

Adams CA, Warnes GM, Nicholas DJD. 1971. A sulfite-dependent nitrate reductase from *Thiobacillus denitrificans*. Biochim Biophys Acta 235:398–406.

Aeckersberg F, Bak F, Widdel F. 1991. Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. Arch Microbiol 156:5–14.

Akagi JM, Chan M, Adams V. 1974. Observations on the bisulfite reductase (P582) isolated from *Desulfotomaculum nigrificans*. J Bacteriol 120:240–244.

Aleem MIH. 1965. Thiosulfate oxidation and electron transport in *Thiobacillus novellus*. J Bacteriol 90:95–101.

Aminuddin M. 1980. Substrate level versus oxidative phosphorylation in the generation of ATP in *Thiobacillus denitrificans*. Arch Microbiol 128:19–25.
Aminuddin M, Nicholas DJD. 1973. Sulfide oxidation linked to the reduction of nitrate to nitrite in *Thiobacillus denitrificans*. Biochim Biophys Acta 325:81–93.
Aminuddin M, Nicholas DJD. 1974a. An AMP-independent sulfite oxidase from *Thiobacillus denitrificans*. J Gen Microbiol 82:103–113.
Aminuddin M, Nicholas DJD. 1974b. Electron transfer during sulfide to sulfite oxidation in *Thiobacillus denitrificans*. J Gen Microbiol 82:115–123.
Arp AJ, Childress JJ. 1983. Sulfide binding by the blood of the hydrothermal vent tube worm *Riftia pachyptila*. Science 219:295–297.
Baalsrud K, Baalsrud KS. 1954. Studies on *Thiobacillus denitrificans*. Arch Mikrobiol 20:34–62.
Baas Becking LGM, Kaplan IR. 1956. The microbiological origin of the sulfur nodules of Lake Eyre. Trans Roy Soc Aust 79:62–65.
Badziong W, Thauer RK. 1978. Growth yields and growth rates of *Desulfovibrio vulgaris* (Marburg) growing on hydrogen plus sulfate and hydrogen plus thiosulfate as sole energy sources. Arch Microbiol 117:209–214.
Badziong W, Thauer RK, Zeikus JG. 1978. Isolation and characterization of *Desulfovibrio* growing on hydrogen plus sulfate as the sole energy source. Arch Microbiol 116:41–49.
Bak F, Cypionka H. 1987. A novel type of energy metabolism involving fermentation of inorganic sulfur compounds. Nature (Lond) 326:891–892.
Bak F, Pfennig N. 1987. Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. Arch Microbiol 147:184–189.
Bak F, Widdel F. 1986a. Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., spec. nov. Arch Microbiol 146:170–176.
Bak F, Widdel F. 1986b. Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum* sp. nov. Arch Microbiol 146:177–180.
Belkin S, Wirsen CO, Jannasch HW. 1986. A new sulfur-reducing, extremely thermophilic eubacterium from a submarine thermal vent. Appl Environ Microbiol 51:1180–1185.
Beudecker RF, Cannon GC, Kuenen JG, Shively JM. 1980. Relations between D-ribulose-1,5-bisphosphate carboxylase, carboxysomes and CO_2 fixing capacity in the obligate chemolithotroph *Thiobacillus neapolitanus* grown under different limitations in the chemostat. Arch Microbiol 124:185–189.
Beudecker RF, Codd GA, Kuenen JG. 1981. Quantification and intracellular distribution of ribulose-1,5-bisphosphate carboxylase in *Thiobacillus neapolitanus*, as related to possible functions of carboxysomes. Arch Microbiol 129:361–367.
Biebl H, Pfennig N. 1977. Growth of sulfate-reducing bacteria with sulfur as electron acceptor. Arch Microbiol 112:115–117.
Bonch-Osmolovskaya EA. 1994. Bacterial sulfur reduction in hot vents. FEMS Microbiol Rev 15:65–77.
Bonch-Osmolovskaya EA, Miroshnichenko ML, Kostrikina NA, Chernych NA, Zavarzin GA. 1990. *Thermoproteus uzoniensis* sp. nov., a new extremely thermophilic archaebacterium from Kamchatka continental hot springs. Arch Microbiol 154:556–559.

Boopathy R, Daniels L. 1991. Isolation and characterization of a furfural degrading sulfate-reducing bacterium from an anaerobic digester. Curr Microbiol 23:327–332.

Böttcher ME, Parafiniuk J. 1998. Methane-derived carbonates in a native sulfur deposit: Stable isotope and trace element discriminations related to the transformation of aragonite to calcite. Isotopes Environ Health Stud 34:177–190.

Böttcher ME, Sievert SM, Kuever J. 1999. Fractionation of sulfur isotopes during dissimilatory reduction of sulfate by a thermophilic gram-negative bacterium at 60°C. Arch Microbiol 172:125–128.

Bowen HJM. 1979. Environmental Chemistry of the Elements. London: Academic Press.

Bowen TJ, Happold FC, Taylor BF. 1966. Studies on adenosine 5′-phosphosulfate reductase from *Thiobacillus denitrificans*. Biochim Biophys Acta 118:566–576.

Brandis A, Thauer RK. 1981. Growth of *Desulfovibrio* species on hydrogen and sulfate as sole energy source. J Gen Microbiol 126:249–252.

Brandis-Heep A, Gebhardt NA, Thauer RK, Widdel F, Pfennig N. 1983. Anaerobic acetate oxidation to CO_2 by *Desulfobacter postgatei*. 1. Demonstration of all enzymes required for the operation of the citric acid cycle. Arch Microbiol 136:222–229.

Brannan DK, Caldwell DE. 1980. *Thermothrix thiopara*: Growth and metabolism of a newly isolated thermophile capable of oxidizing sulfur and sulfur compounds. Appl Environ Microbiol 40:211–216.

Braun M, Stolp H. 1985. Degradation of methanol by a sulfate reducing bacterium. Arch. Microbiol 142:77–80.

Brierley JA. 1966. Contribution of chemoautotrophic bacteria to the acid thermal waters of the Geyser Springs group in Yellowstone Park. PhD Thesis. Montana State Univ, Bozeman.

Brierley CL, Brierley JA. 1973. A chemoautotrophic and thermophilic microorganism isolated from an acid hot spring. Can J Microbiol. 19:183–188.

Brock TD. 1978. Thermophilic Microorganisms and Life at High Temperatures. New York: Springer-Verlag.

Brock TD, Gustafson J. 1976. Ferric iron reduction by sulfur- and iron-oxidizing bacteria. Appl Environ Microbiol 32:567–571.

Brock TD, Madigan MT. 1988. Biology of Microorganisms. 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Brock TD, Brock ML, Bott TL, Edwards MR. 1971. Microbial life at 90°C: The sulfur bacteria of Boulder Springs. J Bacteriol 107:303–314.

Brock TD, Brock KM, Belly RT, Weiss RL. 1972. *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch Microbiol 84:54–68.

Bromfield SM. 1953. Sulfate reduction in partially sterilized soil exposed to air. J Gen Microbiol 8:378–390.

Bryant RD, McGroarty KM, Costerton JW, Laishley EJ. 1983. Isolation and characterization of a new acidophilic *Thiobacillus* species (T. albertis). Can J Microbiol 29:1159–1170.

Burggraf S, Jannasch HW, Nicolaus B, Stetter KO. 1990. *Archeoglobus profundus* sp. nov. represents a new species within the sulfate-reducing archaebacteria. Syst Appl Microbiol 13:24–28.

Burton SD, Morita RY. 1964. Effect of catalase and cultural conditions on growth of Beggiatoa. J Bacteriol 88:1755–1761.

Butlin K. 1953. The bacterial sulfur cycle. Research 6:184–191.
Butlin KR, Postgate JR. 1952. The microbiological formation of sulfur in the Cyrenaikan Lakes. In: Cloudsley-Thompson J, ed. Biology of Deserts. London, UK: Institute of Biology pp 112–122.
Caldwell DE, Caldwell SJ, Laycock JP. 1976. *Thermothrix thiopara* gen. et spec. nov., a facultatively anaerobic, facultatively chemolithotroph living at neutral pH and temperatures. Can J Microbiol 22:1509–1517.
Canfield DD, Des Marais DJ. 1991. Aerobic sulfate reduction in microbial mats. Science 251:1471–1473.
Canfield DE, Thamdrup B. 1994. The production of ^{34}S-depleted sulfide during bacterial disproportionation of elemental sulfur. Science 266:1973–1975.
Cavanaugh CM. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulfide-rich habitats. Nature (Lond) 302:58–61.
Cavanaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterbury JB. 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic symbionts. Science 213:340–342.
Chambers LA, Trudinger PA. 1975. Are thiosulfate and trithionate intermediates in dissimilatory sulfate reduction? J Bacteriol 123:36–40.
Charles AM, Suzuki I. 1966. Mechanism of thiosulfate oxidation by *Thiobacillus novellus*. Biochim Biophys Acta 128:510–521.
Claassen PAM, van den Heuvel MHMJ, Zehnder AJB. 1987. Enzyme profiles of *Thiobacillus versutus* after aerobic and denitrifying growth: Regulation of isocitrate lyase. Arch Microbiol 147:30–36.
Cohen Y, Padan E, Shilo M. 1975. Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. J Bacteriol 123:855–861.
Cohen Y, Krumbein WE, Goldberg M, Shilo M. 1977a. Solar Lake (Sinai). 1. Physical and chemical limnology. Limnol Oceanogr 22:597–608.
Cohen Y, Krumbein WE, Shilo M. 1977b. Solar Lake (Sinai). 2. Distribution of photosynthetic microorganisms and primary production. Limnol Oceanogr 22:609–620.
Cohen Y, Krumbein WE, Shilo M. 1977c. Solar Lake (Sinai). 4. Stromatolitic cyanobacterial mats. Limnol Oceanogr 22:635–656.
Coleman ML, Hedrick DB, Lovley DR, White DC, Pye K. 1993. Reduction of Fe(III) in sediments by sulphate-reducing bacteria. Nature (Lond) 361:436–438.
Corbett CM, Ingledew WJ. 1987. Is Fe^{3+}/Fe^{2+} cycling an intermediate in sulfur oxidation by *Thiobacillus ferrooxidans*? FEMS Microbiol Lett 41:1–6.
Cypionka H, Pfennig N. 1986. Growth yields of *Desulfotomaculum orientis* with hydrogen in chemostat culture. Arch Microbiol 143:396–399.
Daalsgaard T, Bak F. 1992. Effect of acetylene on nitrous oxide reduction and sulfide oxidation in batch and gradient cultures of *Thiobacillus denitrificans*. Appl Environ Microbiol 58:1601–1608.
Davis EA, Johnson EJ. 1967. Phosphorylation coupled to the oxidation of sulfide and 2-mercaptoethanol in extracts of *Thiobacillus thioparus*. Can J Microbiol 13:873–884.
Detmers J, Brüchert V, Habicht KS, Kuever J. 2001. Diversity of sulfur isotope fractionations by sulfate-reducing prokaryotes. Appl Environ Microbiol 67:888–894.

DeWeerd KA, Mandelco L, Tanner RS, Woese CR, Suflita JM. 1990. *Desulfomonile tiedje* gen. nov. spec. nov., a novel anaerobic dehalogenating, sulfate-reducing bacterium. Arch Microbiol 154:23–30.

DeWeerd KA, Concannon F, Suflita JM. 1991. Relationship between hydrogen consumption, dehalogenation, and the reduction of sulfur oxyanions by *Desulfomonile tiedjei*. Appl Environ Microbiol 57:1929–1934.

Dilling W, Cypionka H. 1990. Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol Lett 71:123–128.

Doemel WN, Brock TD. 1974. Bacterial stromatolites: Origin of laminations. Science 184:1083–1085.

Doemel WN, Brock TD. 1976. Vertical distribution of sulfur species in benthic algal mats. Limnol Oceanogr 21:237–244.

Doemel WN, Brock TD. 1977. Structure, growth and decomposition of laminated algal-bacterial mats in alkaline hot springs. Appl Environ Microbiol 34:433–452.

Douglas S, Douglas DD. 2000. Environmental scanning electron microscopy studies of colloidal sulfur deposition in a natural microbial community from a cold sulfide spring near Ancaster, Ontario, Canada. Geomicrobiol J 17:275–289.

Drake HL, Akagi JM. 1978. Dissimilatory reduction of bisulfite by *Desulfovibrio vulgaris*. J Bacteriol 136:916–923.

Dworkin M, editor-in-chief. 2001. The Prokaryotes. Electronic version. New York: Springer-Verlag.

Easterbrook KB, Coombs RW. 1976. Spinin: The subunit protein of bacterial spinae. Can J Microbiol 23:438–440.

Edwards KJ, Bond PL, Gihring TM, Banfield JF. 2000. A new iron-oxidizing, extremely acidophilic archaea is implicated in acid mine drainage generation. Science 287:1796–1799.

Egorova AA, Deryugina ZP. 1963. The spore forming thermophilic thiobacterium: *Thiobacillus thermophilica* Imschenetskii nov. spec. Mikrobiologiya 32:439–446.

Ehrlich GG, Schoen R. 1967. Possible role of sulfur-oxidizing bacteria in surficial acid alteration near hot springs. US Geol Survey Prof Paper 575C, pp C110–C112.

Ehrlich HL. 1999. Microbes as geologic agents: Their role in mineral formation. Geomicrobiol J 16:135–153.

Ehrlich HL, Fox SI. 1967. Copper sulfide precipitation by yeasts from acid mine-waters. Appl Microbiol 15:135–139.

Elsgaard L, Jørgensen BB. 1992. Anoxic transformations of radiolabeled hydrogen sulfide in marine and freshwater sediments. Geochim Cosmochim Acta 56:2425–2435.

Fauque G, Lino AR, Czechowski M, Kang L, DerVertanian DV, Moura JJG, LeGall J, Mora I. 1990. Purification and characterization of bisulfite reductase (desulfofuscidin) from *Desulfovibrio thermophilus* and its complexes with exogenous ligands. Biochim Biophys Acta 1040:112–118.

Fauque G, LeGall J, Barton LL. 1991. Sulfate-reducing and sulfur-reducing bacteria. In: Shively JM, Barton LL, ed. Variations in Autotrophic Life. London, UK: Academic Press, pp 271–337.

Felbeck H. 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, *Riftia pachyptila* Jones (Vestimentifera). Science 213:336–338.

Felbeck H, Childress JJ, Somero GN. 1981. Calvin–Benson cycle and sulfide oxidation enzymes in animals from sulfide-rich habitats. Nature (Lond) 293:291–293.

Fliermans CB, Brock TD. 1972. Ecology of sulfur-oxidizing bacteria in hot acid soils. J Bacteriol 111:343–350.

Freney JR. 1967. Sulfur-containing organics. In: McLaren AD, Petersen GH, eds. Soil Biochemistry. New York: Marcel Dekker, pp 229–259.

Friedrich CG, Rother D, Bardischewsky F, Quentmeier A, Fischer J. 2001. Oxidation of reduced inorganic sulfur compounds by bacteria: Emergence of a common mechanism? Appl Environ Microbiol 67:2873–2882.

Fründ C, Cohen Y. 1992. Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl Environ Microbiol 58:70–77.

Fukui M, Teske A, Assmus B, Muyzer G, Widdel F. 1999. Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus *Desulfonema*) Arch Microbiol 172:193–203.

Garlick S, Oren A, Padan E. 1977. Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. J Bacteriol 129:623–629.

Gebhardt NA, Linder D, Thauer RK. 1983. Anaerobic oxidation of CO_2 by Desulfobacter postgatei. 2. Evidence from ^{14}C-labelling studies for the operation of the citric acid cycle. Arch Microbiol 136:230–233.

Gebhardt NA, Thauer RK, Linder D, Kaulfers P-M, Pfennig N. 1985. Mechanism of acetate oxidation to CO_2 with elemental sulfur in *Desulfuromonas acetoxidans*. Arch Microbiol 141:392–398.

Gevertz D, Telang AJ, Voodrouw G, Jenneman GE. 2000. Isolation and characterization of strains CVO and FWKO B, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. Appl Environ Microbiol 66:2491–2501.

Goffredi SK, Childress JJ, Desaulniers NT, Lallier FH. 1997. Sulfide acquisition by the vent worm *Riftia pachyptila* appears to be via uptake of HS^-, rather than H_2S. J Exp Biol 200(pt 20):2609–2616.

Goldschmidt VM. 1954. Geochemistry. Oxford, UK: Clarendon Press, pp 621–642.

Golovacheva RS, Karavaiko GI. 1978. *Sulfobacillus*, a new genus of thermophilic sporeforming bacteria. Mikrobiologiya 47:815–822 (Engl transl, pp 658–665).

Grayston SJ, Wainwright M. 1988. Sulfur oxidation by soil fungi including species of mycorrhizae and wood-rotting basidiomycetes. FEMS Microbiol Ecol 53:1–8.

Guittonneau G. 1927. Sur l'oxidation microbienne du soufre au cours de l'ammonisation. CR Acad Sci (Paris) 184:45–46.

Guittonneau G, Keiling J. 1927. Sur la solubilisation du soufre élémentaire et la formation des hyposulfides dans une terre riche en azote organique. CR Acad Sci (Paris) 184:898–901.

Hallberg KB, Lindström EB. 1994. Characterization of *Thiobacillus caldus*, sp. nov., a moderately thermophilic acidophile. Microbiology (Reading) 140:3451–3456.

Hallberg KB, Dopson M, Lindström EB. 1996. Reduced sulfur compound oxidation by *Thiobacillus caldus*. J Bacteriol 178:6–11.

Hansen TA, van Gemerden H. 1972. Sulfide utilization by purple sulfur bacteria. Arch Microbiol 86:49–56.

Hansen TA, Veldkamp H. 1973. *Rhodopseudomonas sulfidophila* nov. spec., a new species of the purple nonsulfur bacteria. Arch Microbiol 92:45–58.

Harrison AG, Thode H. 1957. The kinetic isotope effect in the chemical reduction of sulfate. Trans Faraday Soc 53:1–4.

Harrison AG, Thode H. 1958. Mechanism of the bacterial reduction of sulfate from isotope fractionation studies. Trans Faraday Soc. 54:84–92.

Harrison GI, Laishley EJ, Krouse HR. 1980. Stable isotope fractionation by *Clostridium pasteurianum*. 3. Effect of SeO_3^{2-} on the physiology of associated sulfur isotope fractionation during SO_3^{2-} and SO_4^{2-} reductions. Can J Microbiol 26:952–958.

Harrison G, Curle C, Laishley EJ. 1984. Purification and characterization of an inducible dissimilatory type of sulfite reductase from *Clostridium pasteurianum*. Arch Microbiol 138:172–178.

Hoefs J. 1997. Stable Isotope Geochemistry. 4th ed. Berlin: Springer.

Holt JG, ed. 1984. Bergey's Manual of Systematic Bacteriology. Vol 1. Baltimore, MD: Williams & Wilkins.

Holt JG, ed. 1989. Bergey's Manual of Systematic Bacteriology, Vol. 3. Baltimore, MD: Williams & Wilkins.

Holthuijzen YA, van Breemen JFL. Konings WN, van Bruggen EFJ. 1986a. Electron microscopic studies of carboxysomes of *Thiobacillus neapolitanus*. Arch Microbiol 144:258–262.

Holthuijzen YA, van Breemen JFL, Kuenen JG, Konings WN. 1986b. Protein composition of the carboxysomes of *Thiobacillus neapolitanus*. Arch Microbiol 144:398–404.

Holthuijzen YA, van Dissel-Emiliani FFM, Kuenen JG, Konings WN. 1987. Energetic aspects of CO_2 uptake in *Thiobacillus neapolitanus*. Arch Microbiol 147:285–290.

Huber R, Kristjansson JK, Stetter KO. 1987. *Pyrobacylum* gen. nov., a new genus of neutrophilic, rod-shaped archaebacteria from continental solfataras growing optimally at 100°C. Arch Microbiol 149:95–101.

Imhoff-Stuckle D, Pfennig N. 1983. Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. Arch Microbiol 136:194–198.

Isaksen MF, Teske A. 1996. *Desulforhopalus vacuolatus* gen. nov., spec. nov., a new moderately psychrophilic sulfate-reducing bacterium with gas vacuoles isolated from a temperate estuary. Arch Microbiol 166:160–168.

Ivanov MV. 1968. Microbiological Processes in the Formation of Sulfur Deposits. Israel Program for Scientific Translations. Washington, DC: US Dep Agriculture and Natl Sci Foundation.

Jackson BE, McInerney MJ. 2000. Thiosulfate disproportionation by *Desulfotomaculum thermobenzoicum*. Appl Environ Microbiol 66:3650–3653.

Jannasch HW. 1984. Microbial processes at deep sea hydrothermal vents. In: Rona PA, Bostrom K, Laubier L, Smith KL Jr, eds. Hydrothermal Processes at Sea Floor Spreading Centers. New York: Plenum, pp 677–709.

Jannasch HW, Mottl MJ. 1985. Geomicrobiology of deep-sea hydrothermal vents. Science 229:717–725.

Jannasch HW, Taylor CD. 1984. Deep-sea microbiology. Annu Rev Microbiol 38:487–514.

Jannasch HW, Wirsen CO, Molyneaux SJ, Langworthy TA. 1988a. Extremely thermophilic fermentative archaebacteria of the genus *Desulfurococcus* from deep-sea hydrothermal vents. Appl Environ Microbiol 54:1203–1209.

Jannasch HW, Huber R, Belkin S, Stetter KO. 1988b. *Thermotoga neapolitana* sp. nov. of the extremely thermophilic, eubacterial genus *Thermotoga*. Arch Microbiol 150:103–104.

Jannasch HW, Nelson DC, Wirsen CO. 1989. Massive natural occurrence of unusually large bacteria (*Beggiatoa* sp.) at a hydrothermal deep-sea vent site. Nature (Lond) 342:834–836.

Jansen K, Thauer RK, Widdel F, Fuchs G. 1984. Carbon assimilation pathways in sulfate reducing bacteria. Formate, carbon dioxide, carbon monoxide, and acetate assimilation by *Desulfovibrio baarsii*. Arch Microbiol 138:257–262.

Janssen PH, Morgan HW. 1992. Heterotrophic sulfur reduction by *Thermotoga* sp. strain FjSS3B1. FEMS Microbiol Lett 96:213–218.

Janssen PH, Schink B. 1995. Metabolic pathways and energetics of the acetone-oxidizing, sulfate-reducing bacterium, *Desulfobacterium cetonicum*. Arch Microbiol 163:188–194.

Janssen PH, Schuhmann A, Bak F, Liesack W. 1996. Disproportionation of inorganic sulfur compounds by the sulfate-reducing bacterium *Desulfocapsa thiozymogenes* gen. nov., spec. nov. Arch Microbiol 166:184–192.

Jensen ML. 1968. Isotopic geology and the origin of Gulf Coast and Sicilian sulfur deposits. In: International Conference on Saline Deposits, 1962. Special Paper 88. Boulder, CO: Geol Soc of Am, pp 525–536.

Jones GE, Starkey RL. 1957. Fractionation of stable isotopes of sulfur by microorganisms and their role in deposition of native sulfur. Appl Microbiol 5:111–118.

Jones GE, Starkey RL. 1962. Some necessary conditions for fractionation of stable isotopes of sulfur by *Desulfovibrio desulfuricans*. In: Jensen ML, ed. Biogeochemistry of Sulfur Isotopes. NSF Symp. New Haven, CT: Yale Univ Press, pp 61–79.

Jones GE, Starkey RL, Feeley HW, Kulp JL. 1956. Biological origin of native sulfur in salt domes of Texas and Louisiana. Science 123:1124–1125.

Jones ML. 1981. *Riftia pachyptila* Jones: Observations on the vestimentiferan worm from the Galápagos Rift. Science 213:333–336.

Jørgensen BB. 1990a. A thiosulfate shunt in the sulfur cycle of marine sediments. Science 249:152–154.

Jørgensen BB. 1990b. The sulfur cycle of freshwater sediments: Role of thiosulfate. Limnol Oceanogr 35:1329–1342.

Jørgensen BB, Bak F. 1991. Pathways of microbiology of thiosulfate transformations and sulfate reduction in marine sediment (Kattegat, Denmark). Appl Environ Microbiol 57:847–856.

Jørgensen BB, Kuenen JG, Cohen Y. 1979a. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol Oceanogr 24:799–822.

Jørgensen BB, Revsbech NP, Blackburn H, Cohen Y. 1979b. Diurnal cycle of oxygen and sulfide microgradient and microbial photosynthesis in a cyanobacterial mat sediment. Appl Environ Microbiol 38:46–58.

Jørgensen BB, Isaksen MF, Jannasch HW. 1992. Bacterial sulfate reduction above 100°C in deep-sea hydrothermal vent sediments. Science 258:1756–1757.

Justin P, Kelly DP. 1978. Growth kinetics of *Thiobacillus denitrificans* in anerobic and aerobic chemostat culture. J Gen Microbiol 107:123–130.

Kaplan IR, Rittenberg SC. 1962. The microbiological fractionation of sulfur isotopes. In: Jensen ML, ed. Biogeochemistry of Sulfur Isotopes. NSF Symp. New Haven, CT: Yale Univ Press, pp 80–93.

Kaplan IR, Rittenberg SC. 1964. Microbiological fractionation of sulfur isotopes. J Gen Microbiol 34:195–212.

Kaplan IR, Rafter TA, Hulston JR. 1960. Sulfur isotopic variations in nature. Part 8. Application to some biochemical problems. NZ J Sci 3:338–361.

Karavaiko GI, Golyshina OV, Troitskii AV, Valicho-Roman KM, Golovacheva RS, Pivovarova TA. 1994. *Sulfurococcus yellowstonii* sp. nov., a new species of iron- and sulfur-oxidizing thermoacidophilic archaebacteria. Mikrobiologiya 63:668–682 (Engl transl. pp 379–387).

Keith SM, Herbert RA. 1983. Dissimilatory nitrate reduction by a strain of *Desulfovibrio desulfuricans*. FEMS Microbiol Lett 18:55–59.

Kelly DP. 1982. Biochemistry of the chemolithotrophic oxidation of inorganic sulfur. Phil Trans Roy Soc Lond B 298:499–528.

Kelly DP, Wood AP. 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. Int J Syst Evol Microbiol 50:511–516.

Kelly DP, Lu W-P, Poole RK. 1993. Cytochromes in *Thiobacillus tepidarius* and the respiratory chain involved in the oxidation of thiosulfate and tetrathionate. Arch Microbiol 160:87–95.

Kemp ALW, Thode HG. 1968. The mechanism of the bacterial reduction of sulfate and sulfite from isotope fractionation studies. Geochim Cosmochim Acta 32:71–91.

Kobiyashi KS, Tashibana S, Ishimoto M. 1969. Intermediary formation of trithionate in sulfite reduction by a sulfate-reducing bacterium. J Biochem (Tokyo) 65:155–157.

Kobiyashi K, Tukahashi E, Ishimoto M. 1972. Biochemical studies on sulfate-reducing bacteria. XI. Purification and some properties of sulfite reductase, desulfoviridin. J Biochem (Tokyo) 72:879–887.

Kramer JF, Pope DH, Salerno JC. 1987. Pathways of electron transfer in *Desulfovibrio*. In: Kim CH, Tedeschi H, Diwan JJ, Salerno SC, eds. Advances in Membrane Biochemistry and Bioenergetics. New York: Plenum Press, pp 249–258.

Krekeler D, Sigalevich P, Teske A, Cypionka H, Cohen Y. 1997. A sulfate-reducing bacterium from the oxic layer of a microbial mat from Solar Lake (Sinai), *Desulfovibrio oxyclinae* sp. nov. Arch Microbiol 167:369–375.

Kuenen JG, Beudecker RF. 1982. Microbiology of thiobacilli and other sulfur-oxidizing autotrophs, mixotrophs, and heterotrophs. Phil Trans Roy Soc Lond B 298:473–497.

Kuenen JG, Tuovinen OH. 1981. The genera *Thiobacillus* and *Thiomicrospira*. In: Starr MP, Stolp H, Tüper HG, Ballows A, Schlegel H, eds. The Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria. Berlin, Germany: Springer-Verlag, pp 1023–1036.

Kuever J, Kulmer J, Jannsen S, Fischer U, Blotevogel K-H. 1993. Isolation and characterization of a new spore-forming sulfate-reducing bacterium growing by complete oxidation of catechol. Arch Microbiol 159:282–288.

Laishley EJ, Krouse HR. 1978. Stable isotope fractionation by *Clostridium pasteurianum*. 2. Regulation of sulfite reductases by sulfur amino acids and their influence on sulfur isotope fractionation during SO_3^{2-} and SO_4^{2-} reduction. Can J Microbiol 24: 716–724.

LaRiviere JWM, Schmidt K. 1981. Morphologically conspicuous sulfur-oxidizing bacteria. In: Starr MP, Stolp H, Tüper HG, Ballows A, Schlegel H, eds. The

Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria. Berlin, Germany: Springer-Verlag, pp 1037–1048.
Lawton K. 1955. Chemical composition of soils. In: Bear FE, ed. Chemistry of the Soil. New York: Reinhold pp 53–84.
Lee, JP, Peck HD Jr. 1971. Purification of the enzyme reducing bisulfite to trithionate from *Desulfovibrio gigas* and its identification as desulfoviridin. Biochem Biophys Res Commun 45:583–589.
Lee JP, Yi CS, LeGall J, Peck HD. 1973. Isolation of a new pigment, desulfoviridin, from *Desulfovibrio desulfuricans* (Norway strain) and its role in sulfite reduction. J Bacteriol 115:453–455.
LeRoux N, Wakerley DS, Hunt SD. 1977. Thermophilic thiobacillus-type bacteria from Icelandic thermal areas. J Gen Microbiol 100:197–201.
Liu C-L, Hart N, Peck HD Jr. 1982. Inorganic pyrophosphate: Energy source for sulfate-reducing bacteria of the genus *Desulfotomaculum*. Science 217:363–364.
Ljunggren P. 1960. A sulfur mud deposit formed through bacterial transformation of fumarolic hydrogen sulfide. Econ Geol Bull Soc Econ Geol 55:531–538.
London J. 1963. *Thiobacillus intermedius* nov. sp. a novel type of facultative autotroph. Arch Microbiol 46:329–337.
London J, Rittenberg SC. 1964. Path of sulfur in sulfide and thiosulfate oxidation by thiobacilli. Proc Natl Acad Sci USA 52:1183–1190.
London J, Rittenberg SC. 1966. Effects of organic matter on the growth of *Thiobacillus intermedius*. J Bacteriol 91:1062–1069.
London J, Rittenberg SC. 1967. *Thiobacillus perometabolis* nov. sp., a non-autotrophic thiobacillus. Arch Microbiol 59:218–225.
Londry KL, Suflita JM, Tanner RS. 1999. Cresol metabolism by the sulfate-reducing bacterium *Desulfotomaculum* sp. strain Groll. Can J Microbiol 45:458–463.
Lovley DR, Roden EE, Phillips EJP, Woodward JC. 1993. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar Geol 113:41–53.
Loya S, Yanofsky SA, Epel BL. 1982. Characterization of cytochromes in lithotrophically and organotrophically grown cells of *Thiobacillus* A_2. J Gen Microbiol 128:2371–2378.
Lu W-P. 1986. A periplasmic location for the bisulfite-oxidizing multienzyme system from *Thiobacillus versutus*. FEMS Microbiol Lett 34:313–317.
Lu W-P, Kelly DP. 1983. Purification and some properties of two principal enzymes of the thiosulfate-oxidizing multienzyme system from *Thiobacillus* A_2. J Gen Microbiol 129:3549–3562.
Lu W-P, Kelly DP. 1988. Respiration-driven proton translocation in *Thiobacillus versutus* and the role of the periplasmic thiosulfate-oxidizing system. Arch Microbiol 149:297–302.
Ma K, Weiss R, Adams MWW. 2000. Characterization of hydrogenase II from the hyperthermophilic archeon *Pyrococcus furiosus* and assessment of its role in sulfur reduction. J Bacteriol 182:1864–1871.
Macnamara J, Thode H. 1951. The distribution of ^{34}S in nature and the origin of native sulfur deposits. Research 4:582–583.
Madigan MT, Brock TD. 1975. Photosynthetic sulfide oxidation by *Chloroflexus aurantiacus*, a filamentous, photosynthetic, gliding bacterium. J Bacteriol, 122:782–784.

Markosyan GE. 1973. A new mixotrophic sulfur bacterium developing in acidic media, *Thiobacillus organoparus* sp. n. Dokl Akad Nauk SSSR Ser Biol 211:1205–1208.

Marshall C, Frenzel P, Cypionka H. 1993. Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. Arch Microbiol 159:168–173.

Martinez JD. 1991. Salt domes. Am Sci 79:420–431.

Mason J, Kelly DP. 1988. Mixotrophic and autotrophic growth of *Thiobacillus acidophilus* on tetrathionate. Arch Microbiol 149:317–323.

McCready RGL, Laishley EJ, Krouse HR. 1975. Stable isotope fractionation by *Clostridium pasteurianum*. 1. $^{34}S/^{32}S$: Inverse isotope effects during SO_4^{2-} and $SO_3^{22/Mac}$ reduction. Can J Microbiol 21:235–244.

McCready RGL, Gould WD, Cook FD. 1983. Respiratory nitrate reduction by *Desulfovibrio* sp. Arch Microbiol 135:182–185.

Mechalas BJ, Rittenberg SC. 1960. Energy coupling in *Desulfovibrio desulfuricans*. J Bacteriol 80:501–507.

Meckenstock RU, Annweiler E, Michaelis W, Richnow HH, Schink B. 2000. Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. Appl Environ Microbiol 66:2743–2747.

Meulenberg R, Pronk JT, Hazeu W, van Dijken JP, Frank J, Bos P, Kuenen JG. 1993. Purification and partial characterization of thiosulfate dehydrogenase from *Thiobacillus acidophilus*. J Gen Microbiol 139:2033–2039.

Milhaud G, Aubert JP, Millet J. 1958. Role physiologique du cytochrome C de la bacterie chemiautotrophe *Thiobacillus denitrificans*. CR Acad Sci (Paris) 246:1766–1769.

Miller JDA, Wakerley DS. 1966. Growth of sulfate-reducing bacteria by fumarate dismutation. J Gen Microbiol 43:101–107.

Minz D, Fishbain S, Green SJ, Muyzer G, Cohen Y, Rittman BE, Stahl DA. 1999a. Unexpected population distribution in a microbial mat community: Sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl Environ Microbiol 65:4659–4665.

Minz D, Flax JL, Green SJ, Muyzer G, Cohen Y, Wagner M, Rittman BE, Stahl DA. 1999b. Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl Environ Microbiol 65:4666–4671.

Mitchell GJ, Jones JG, Cole JA. 1986. Distribution and regulation of nitrate and nitrite reduction by *Desulfovibrio* and *Desulfotomaculum* species. Arch Microbiol 144:35–40.

Möller D, Schauder R, Fuchs G, Thauer RK. 1987. Acetate oxidation to CO_2 via a citric acid cycle involving an ATP-citrate lyase: A mechanism for the synthesis of ATP via substrate level phosphorylation in *Desulfobacter postgatei* growing on acetate and sulfate. Arch Microbiol 148:202–207.

Moriarty DJW, Nicholas DJD. 1970. Electron transfer during sulfide and sulfite oxidation by *Thiobacillus concretivorus*. Biochem Biophys Acta 216:130–138.

Mosser JL, Mosser AG, Brock TD. 1973. Bacterial origin of sulfuric acid in geothermal habitats. Science 179:1323–1324.

Mosser JL, Bohlool BB, Brock TD. 1974. Growth rates of *Sulfolobus acidocaldarius* in nature. J Bacteriol 118:1075–1081.

Nathansohn A. 1902. Über eine neue Gruppe von Schwefelbakterien und ihren Stoffwechsel. Mitt Zool Sta Neapel 15:655–680.

Nelson DC, Castenholz RW. 1981. Use of reduced sulfur compounds by *Beggiatoa* sp. J Bacteriol 147:140–154.

Nelson DC, Jannasch HW. 1983. Chemoautotrophic growth of a marine *Beggiatoa* in sulfide-gradient cultures. Arch Microbiol 136:262–269.

Neutzling O, Pfleiderer C, Trüper HG. 1985. Dissimilatory sulfur metabolism in phototrophic "non-sulfur" bacteria. J Gen Microbiol 131:791–798.

Nübel T, Klughammer C, Huber R, Hauska G, Schütz M. 2000. Sulfide: quinone oxidoreductase in membranes of the hyperthermophilic bacterium *Aquifex aeolicus* (VF5). Arch Microbiol 173:233–244.

Odom JM, Peck HD Jr. 1981. Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria, *Desulfovibrio* sp. FEMS Microbiol Lett 12:47–50.

Odom JM, Wall JD. 1987. Properties of a hydrogen-inhibited mutant of *Desulfovibrio desulfuricans* ATCC 27774. J Bacteriol 169:1335–1337.

Oren A, Shilo M. 1979. Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*: Sulfur respiration and lactate fermentation. Arch Microbiol 122:77–84.

Ozawa K, Meikari T, Motohashi K, Yoshida M, Akutsu H. 2000. Evidence for the presence of an F-type ATP synthase involved in sulfate respiration in *Desulfovibrio vulgaris*. J Bacteriol 182:2200–2206.

Panganiban AT, Hanson RS. 1976. Isolation of a bacterium that oxidizes methane in the absence of oxygen. Abstr Annu Meet Am Soc Microbiol I59:121.

Panganiban AT, Patt TE, Hart W, Hanson RS. 1979. Oxidation of methane in the absence of oxygen in lake water samples. Appl Environ Microbiol 37:303–309.

Pankhania IP, Spormann AM, Hamilton WA, Thauer RK. 1988. Lactate conversion to acetate, CO_2, and H_2 in cell suspensions of *Desulfovibrio vulgaris* (Marburg): Indications for the involvement of an energy driven reaction. Arch Microbiol 150:26–31.

Paschinger H, Paschinger J, Gaffron H. 1974. Photochemical disproportionation of sulfur into sulfide and sulfate by *Chlorobium limicola* forma *thiosulfatophilum*. Arch Microbiol 96:341–351.

Paulsen J, Kröger A, Thauer RK. 1986. ATP-driven succinate oxidation in the catabolism of *Desulfuromonas acetoxidans*. Arch Microbiol 144:78–83.

Peck HD Jr. 1962. Symposium on metabolism of inorganic compounds. V. Comparative metabolism of inorganic sulfur compounds in microorganisms. Bacteriol Rev 26:67–94.

Peck HD Jr. 1993. Bioenergetic strategies of the sulfate-reducing bacteria. In: Odom JM, Singleton R Jr, eds. The Sulfate-Reducing Bacteria: Contemporary Perspectives. New York: Springer-Verlag, pp 41–76.

Peck HD Jr, LeGall J. 1982. Biochemistry of dissimilatory sulfate reduction. Phil Trans Roy Soc Lond B 298:443–466.

Peeters T, Aleem MIH. 1970. Oxidation of sulfur compounds and electron transport in *Thiobacillus denitrificans*. Arch Microbiol 71:319–330.

Perry KA, Kostka JE, Luther GW III, Nealson KH. 1993. Mediation of sulfur speciation by a Black Sea facultative anaerobe. Science 259:801–803.

Pfennig N. 1977. Phototrophic green and purple bacteria: A comparative, systematic survey. Annu Rev Microbiol 31:275–290.

Pfennig N, Biebl H. 1976. *Desulfuromonas acetoxidans* gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch Microbiol 110:3–12.

Pfennig N, Biebl H. 1981. The dissimilatory sulfur-reducing bacteria. In: Starr MP, Stolp H, Trüper HG, Ballows A, Schlegel H, eds. The Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria, Vol 1. Berlin, Germany: Springer-Verlag, pp 941–947.

Pfennig N, Widdel F, Trüper HG. 1981. The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Trüper HG, Ballows A, Schlegel H, eds. The Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria, Vol 1. Berlin, Germany: Springer-Verlag, pp 926–940.

Pierson BK, Castenholz RW. 1974. A photosynthetic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus* gen. and spec. nov. Arch Microbiol 100:5–24.

Platen H, Temmes A, Schink B. 1990. Anaerobic degradation of acetone by *Desulfurococcus biacutus* spec. nov. Arch Microbiol 154:355–361.

Postgate JR. 1952. Growth of sulfate reducing bacteria in sulfate-free media. Research 5:189–190.

Postgate JR. 1963. Sulfate-free growth of *Cl. nigrificans*. J Bacteriol 85:1450–1451.

Postgate JR. 1984. The Sulfate-Reducing Bacteria. 2nd ed., Cambridge, UK: Cambridge Univ Press.

Powell MA, Somero GN. 1983. Blood components prevent sulfide poisoning of respiration of the hydrothermal vent tube worm *Riftia pachyptila*. Science 219:297–299.

Pringsheim EG. 1967. Die Mixotrophie von *Beggiatoa*. Arch Mikrobiol 59:247–254.

Pronk JT, Meulenberg R, Hazeu W, Bos P, Kuenen JG. 1990. Oxidation of reduced inorganic sulfur compounds by acidophilic thiobacilli. FEMS Microbiol Rev 75:293–306.

Pronk JT, Liem K, Bos P, Kuenen JG. 1991. Energy transduction by anaerobic ferric iron respiration in *Thiobacillus ferrooxidans*. Appl Environ Microbiol 57:2063–2068.

Pronk JT, De Bruyn JC, Bos P, Kuenen JG. 1992. Anaerobic growth of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58:2227–2230.

Qatabi AI, Nivière V, Garcia JL. 1991. *Desulfovibrio alcoholovorans* sp. nov., a sulfate-reducing bacterium able to grow on glycerol, 1,2- and 1,3-propanol. Arch Microbiol 155:143–148.

Rau GH. 1981. Hydrothermal vent clam and tube worm $^{13}C/^{12}C$: Further evidence of nonphotosynthetic food sources. Science 213:338–340.

Rau GH, Hedges JI. 1979. Carbon-13 depletion in a hydrothermal vent mussel: Suggestion of a chemosynthetic food source. Science 203:648–649.

Ravot G, Magot M, Fardeau ML, Patel BKC, Prensier G, Egan A, Garcia JL, Ollivier B. 1995. *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. Int J Syst Bacteriol 45:308–314.

Rees GN, Harfoot CG, Sheehy AJ. 1998. Amino acid degradation by the mesophilic sulfate-reducing bacterium *Desulfobacterium vacuolatum*. Arch Microbiol 169: 76–80.

Roy AB, Trudinger PA. 1970. The Biochemistry of Inorganic Compounds of Sulfur. Cambridge, UK: Cambridge Univ Press.

Rueter P, Rabus R, Wilkes H, Aeckersberg F, Rainey FA, Jannasch HW, Widdel F. 1994. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulfate-reducing bacteria. Nature (Lond) 372:455–458.

Sadler MH, Johnson EJ. 1972. A comparison of the NADH oxidase electron transport system of two obligately chemolithotrophic bacteria. Biochim Biophys Acta 283:167–179.

Schauder R, Kröger A. 1993. Bacterial sulfur respiration. Arch Microbiol 159:491–497.

Schauder R, Müller E. 1993. Polysulfide as a possible substrate for sulfur-reducing bacteria. Arch Microbiol 160:377–382.

Schauder R, Eikmanns B, Thauer RK, Widdel F, Fuchs G. 1986. Acetate oxidation to CO_2 in anaerobic bacteria via a novel pathway not involving reactions of the citric acid cycle. Arch Microbiol 145:162–172.

Schauder R, Widdel F, Fuchs G. 1987. Carbon assimilation pathways in sulfate-reducing bacteria. II. Enzymes of a reductive citric acid cycle in autotrophic *Desulfobacter hydrogenophilus*. Arch Microbiol 148:218–225.

Schauder R, Preuss A, Jetten M, Fuchs G. 1989. Oxidative and reductive acetyl CoA/carbon monoxide dehydrogenase pathway in *Desulfobacterium autotrophicum*. Arch Microbiol 151:84–89.

Schedel M, Tnüper HG. 1980. Anaerobic oxidation of thiosulfate and elemental sulfur in *Thiobacillus denitrificans*. Arch Microbiol 124:205–210.

Schicho RN, Ma K, Adams MWW, Kelly RM. 1993. Bioenergetics of sulfur reduction in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 175:1823–1830.

Schnell S, Schink B. 1991. Anaerobic aniline degradation via reductive deamination of 4-aminobenzoyl-CoA in *Desulfobacterium anilini*. Arch Microbiol 155:183–190.

Schoen R, Ehrlich GG. 1968. Bacterial origin of sulfuric acid in sulfurous hot springs. 23rd Int Geol Congr 17:171–178.

Schoen R, Rye RO. 1970. Sulfur isotope distribution in solfataras, Yellowstone National Park. Science 170:1082–1084.

Segerer A, Neuner A, Kristiansson JK, Stetter KO. 1986. *Acidianus infernus* gen. nov., sp. nov., and *Acidianus brierleyi* comb. nov.: Facultative aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaebacteria. Int J Syst Bacteriol. 36:559–564.

Seitz H-J, Cypionka H. 1986. Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. Arch Microbiol 146:63–67.

Selig M, Schönheit P. 1994. Oxidation of organic compounds to CO_2 with sulfur or thiosulfate as electron acceptor in the anaerobic hyperthermophilic archaea *Thermoproteus tenax* and *Pyrobaculum islandicum* proceeds via the citric acid cycle. Arch Microbiol 162:286–294.

Shively JM, Ball F, Brown DH, Saunders RE. 1973. Functional organelles in prokaryotes: Polyhedral inclusions (carboxysomes) of *Thiobacillus neapolitanus*. Science 182:584–586.

Shivvers DW, Brock TD. 1973. Oxidation of elemental sulfur by *Sulfolobus acidocaldarius*. J Bacteriol 114:706–710.

Shturm LD. 1948. Sulfate reduction by facultative aerobic bacteria. Mikrobiologiya 17:415–418.

Sigalevich P, Cohen Y. 2000. Oxygen-dependent growth of sulfate-reducing bacterium *Desulfovibrio oxyclinae* in coculture with *Marinobacter* sp. strain MB in an aerated sulfate-depleted chemostat. Appl Environ Microbiol 66:5019–5023.

Sigalevich P, Baev MV, Teske A, Cohen Y. 2000a. Sulfate reduction and possible aerobic metabolism of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in a chemostat coculture with *Marinobacter* sp. strain MB under exposure of increasing oxygen concentrations. Appl Environ Microbiol 66:5013–5018.

Sigalevich P, Meshorer E, Helman Y, Cohen Y. 2000b. Transition from anaerobic to aerobic growth conditions for the sulfate-reducing bacterium *Desulfovibrio oxyclinae* results in flocculation. Appl Environ Microbiol 66:5005–5012.

Skerman VBD, Dementyeva G, Carey B. 1957a. Intracellular deposition of sulfur by *Sphaerotilus natans*. J Bacteriol 73:504–512.

Skerman VBD, Dementyeva G, Skyring GW. 1957b. Deposition of sulfur from hydrogen sulfide by bacteria and yeasts. Nature (Lond) 179:742.

Skirnisdottir S, Hreggvidsson GO, Hjörleifsdottir S, Marteinsson VT, Petursdottir SK, Holst O, Kristjansson JK. 2000. Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats. Appl Environ Microbiol 66:2835–2841.

Skyring GW. 1987. Sulfate reduction in coast ecosystems. Geomicrobiol J 5:295–374.

Smith AL, Kelly DP, Wood AP. 1980. Metabolism of *Thiobacillus* A_2 grown under autotrophic, mixotrophic, and heterotrophic conditions in chemostat culture. J Gen Microbiol 121:127–138.

Smith DW, Rittenberg SC. 1974. On the sulfur-source requirement for growth of *Thiobacilllus intermedius*. Arch Microbiol 100:65–71.

Smith DW, Strohl WR. 1991. Sulfur-oxidizing bacteria. In: Shively JM, Barton LL, eds. Variations in Autotrophic Life. London, UK: Academic Press, pp 121–146.

Smock AM, Böttcher ME, Cypionka H. 1998. Fractionation of sulfur isotopes during thiosulfate reduction by *Desulfovibrio desulfuricans*. Arch Microbiol 169:460–463.

Sokolova GA. 1962. Microbiological sulfur formation in Sulfur Lake. Mikrobiologiya 31:324–327 (Engl transl; pp 264–266).

Sokolova GA, Karavaiko GI. 1968. Physiology and Geochemical Activity of Thiobacilli. Engl transl. Springfield, VA: US Dep of Commerce. Clearinghouse Fed Sci Tech Info.

Sorokin YuI. 1966a. Role of carbon dioxide and acetate in biosynthesis of sulfate-reducing bacteria. Nature (Lond) 210:551–552.

Sorokin YuI. 1966b. Sources of energy and carbon for biosynthesis by sulfate-reducing bacteria. Mikrobiologiya 35:761–766 (Engl transl, pp 643–647).

Sorokin YuI. 1966c. Investigation of the structural metabolism of sulfate-reducing bacteria with ^{14}C. Mikrobiologiya 35:967–977 (Engl transl, pp 806–814).

Sorokin YuI. 1966d. The role of carbon dioxide and acetate in biosynthesis in sulfate reducing bacteria. Dokl Akad Nauk SSSR 168:199.

Sorokin YuI. 1970. The mechanism of chemical and biological oxidation of sodium, calcium, and iron sulfides. Mikrobiologiya 39:253–258 (Engl transl, pp 220–224).

Speich N, Trüper HG. 1988. Adenylsulfate reductase in a dissimilatory sulfate-reducing archaebacterium. J Gen Microbiol 134:1419–1425.

Stanier RY, Ingraham JL, Wheelis ML, Painter PR. 1986. The Microbial World. 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Starkey RL. 1934. The production of polythionates from thiosulfate by microorganisms. J Bacteriol 28:387–400.

Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG, eds. 1981. The Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria. Berlin, Germany: Springer-Verlag.

Stetter KO. 1985. Thermophilic archaebacteria occurring in submarine hydrothermal areas. In: Caldwell DE, Brierley JA, Brierley CL, eds. Planetary Ecology. New York: Van Nostrand Reinhold, pp 320–332.

Stetter KO. 1988. *Archeoglobus fulgidus* gen. nov., sp. nov.: A new taxon of extremely thermophilic archaebacteria. Syst Appl Microbiol 10:172–173.

Stetter KO, Koenig H, Stackebrandt E. 1983. *Pyrodictium* gen nov., a new genus of submarine disk-shaped sulfur-reducing archaebacteria growing optimally at 105°C. Syst Appl Microbiol 4:535–551.

Stetter KO, Lauerer G, Thomm M, Neuner A. 1987. Isolation of extremely thermophilic sulfate reducers: Evidence for a novel branch of archaebacteria. Science 236:822–824.

Steuber J, Cypionka H, Kroneck PMH. 1994. Mechanism of dissimilatory sulfite reduction by *Desulfovibrio desulfuricans*: Purification of a membrane-bound sulfite reductase and coupling with cytochrome c_3 and hydrogenase. Arch Microbiol 162:255–260.

Strahler AN. 1977. Principles of Physical Geology. New York: Harper & Row.

Strohl WR, Larkin JM. 1978. Enumeration, isolation, and characterization of *Beggiatoa* from freshwater sediments. Appl Environ Microbiol 36:755–770.

Suzuki I. 1965. Oxidation of elemental sulfur by an enzyme system of *Thiobacillus thiooxidans*. Biochim Biophys Acta 104:359–371.

Suzuki I. 1999. Oxidation of inorganic sulfur compounds: Chemical and enzymatic reactions. Can J Microbiol 45:97–105.

Suzuki I, Silver M. 1966. The initial product and properties of the sulfur-oxidizing enzyme of thiobacilli. Biochim Biophys Acta 122:22–33.

Suzuki I, Chan CW, Takeuchi TL. 1992. Oxidation of elemental sulfur to sulfite by *Thiobacillus thiooxidans* cells. Appl Environ Microbiol 58:3767–3769.

Suzuki I, Chan CW, Takeuchi TL. 1994. Oxidation of inorganic sulfur compounds by Thiobacilli. In: Alpers CN, Blowes DW, eds. Environmental Geochemistry of Sulfide Oxidation. ACS Symp 550. Washington, DC: Am Chem Soc, pp 60–67.

Szewzyk R, Pfennig N. 1987. Complete oxidation of catechol by a strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. Arch Microbiol 147:163–168.

Tasaki M, Kamagata Y, Nakamura K, Mikami E. 1991. Isolation and characterization of a thermophilic benzoate-degrading sulfate-reducing bacterium, *Desulfotomaculum thermobenzoicum* sp. nov. Arch Microbiol 155:348–352.

Tasaki M, Kamagata Y, Nakamura K, Mikami E. 1992. Utilization of methoxylated benzoates and formation of intermediates by *Desulfotomaculum thermobenzoicum* in the presence and absence of sulfate. Arch Microbiol 157:209–212.

Teske A, Brinkhoff T, Muyzer G, Moser DP, Rethmeier J, Jannasch HW. 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. Appl Environ Microbiol 66:3125–3133.

Thamdrup B, Finster K, Hansen JW, Bak F. 1993. Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron and manganese. Appl Environ Microbiol 59:101–108.

Thode HG, Wanless K, Wallouch R. 1954. The origin of native sulfur deposits from isotopic fractionation studies. Geochim Cosmochim Acta 5:286–298.

Trautwein K. 1921. Beitrag zur Physiologie und Morphologie der Thionsäurebakterien. Bakteriol Parasitenk Infektionskr Hyg Abt II 53:513–548.

Trudinger PA. 1961. Thiosulfate oxidation and cytochromes in *Thiobacillus* X. 2. Thiosulfate oxidizing enzyme. Biochem J 78:680–686.

Trudinger PA. 1970. Carbon monoxide-reacting pigment from *Desulfotomaculum nigrificans* and its possible relevance to sulfite oxidation. J Bacteriol 104:158–170.

Trüper HG. 1978. Sulfur metabolism. In: Clayton RK, Sistrom WR, eds. The Photosynthetic Bacteria. New York: Plenum Press, pp 677–690.

Trüper HG. 1984a. Microorganisms and the sulfur cycle. In: Müller A, Krebs B, eds. Sulfur, Its Significance for Chemistry, for the Geo-, Bio-, and Cosmosphere and Technology vol 5. Amsterdam: Elsevier, pp 351–365.

Trüper HG. 1984b. Phototrophic bacteria and the sulfur metabolism. In: Müller A, Krebs B, eds. Sulfur, its Significance for Chemistry, for the Geo-, Bio-, and Cosmosphere and Technology. Vol. 5, Amsterdam: Elsevier, pp 367–382.

Tuttle JH, Ehrlich HL. 1986. Coexistence of inorganic sulfur metabolism and manganese oxidation in marine bacteria. Abstr, Annu Meet Am Soc Microbiol I-21: 168.

Tuttle JH, Holmes PE, Jannasch HW. 1974. Growth rate stimulation of marine pseudomonads by thiosulfate. Arch Microbiol 99:1–14.

Vainshtein MB. 1977. Oxidation of hydrogen sulfide by thionic bacteria. Mikrobiologiya 46:1114–1116 (Engl transl., pp 898–899).

Vainshtein MB, Matrosov AG, Baskunov VB, Zyakun AM, Ivanov MV. 1981. Thiosulfate as in intermediate product of bacterial sulfate reduction. Mikrobiologiya 49:855–859 (Engl transl, pp 672–675).

Vairavamurthy A, Manowitz B, Luther GW III, Jeon Y. 1993. Oxidation state of sulfur in thiosulfate and implications for anaerobic energy metabolism. Geochim. Cosmochim. Acta 57:1619–1623.

van den Ende FP, van Gemerden H. 1993. Sulfide oxidation under oxygen limitation by a *Thiobacillus thioparus* isolated from a marine microbial mat. FEMS Microbiol Ecol 13:69–78.

van Gemerden H. 1986. Production of elemental sulfur by green and purple sulfur bacteria. Arch Microbiol 146:52–56.

Vishniac W. 1952. The metabolism of *Thiobacillus thioparus*. I. The oxidation of thiosulfate. J Bacteriol 64:363–373.

Vishniac W, Santer M. 1957. The thiobacilli. Bacteriol Rev 21:195–213.

Wainer A. 1964. The production of sulfate from cysteine without the formation of free cysteinesulfinic acid. Biochem Biophys Res Commun 16:141–144.

Wainer A. 1967. Mitochondrial oxidation of cysteine. Biochim. Biophys. Acta 141:466–472.

Wall JD, Rapp-Giles BJ, Brown MF, White JA. 1990. Response of *Desulfovibrio desulfuricans* colonies to oxygen stress. Can J Microbiol 36:400–408.

Wedepohl KH. 1984. Sulfur in the Earth's crust, its origin and natural cycle. In: Müller A, Krebs B, eds. Sulfur: Its Significance for Chemistry, for the Geo-, Bio-, and Cosmosphere and Technology, Vol 5, Amsterdam: Elsevier, pp 39–54.

Widdel F, Pfennig N. 1977. A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. Arch Microbiol 112:119–122.

Widdel F, Pfennig N. 1981. Sporulation and further nutritional characteristics of *Desulfotomaculum acetoxidans*. Arch Microbiol 129:401–402.

Williams CD, Nelson DC, Farah BA, Jannasch HW, Shively JM. 1988. Ribulose bisphosphate carboxylase of the procaryotic symbiont of a hydrothermal vent tube worm: Kinetics, activity, and gene hybridization. FEMS Microbiol Lett 50:107–112.

Williams RAD, Hoare DS. 1972. Physiology of a new facultative autotrophic thermophilic *Thiobacillus*. J Gen Microbiol 70:555–566.

Wirsen CO, Jannasch HW. 1978. Physiological and morphological observations on *Thiovulum* sp. J Bacteriol 136:765–774.

Wood AP, Kelly DP. 1983. Autotrophic, mixotrophic and heterotrophic growth with denitrification by *Thiobacillus* A_2 under anaerobic conditions. FEMS Microbiol Lett 16:363–370.

Wood AP, Kelly DP. 1991. Isolation and characterization of *Thiobacillus halophilus* sp. nov., a sulfur-oxidizing autotrophic eubacterium from a Western Australian hypersaline lake. Arch Microbiol 156:277–280.

Wood P. 1988. Chemolithotrophy. In: Anthony C, ed. Bacterial Energy Transduction. London: Academic Press, pp 183–230.

Yamanaka T. 1996. Mechanism of oxidation of inorganic electron donors in autotrophic bacteria. Plant Cell Physiol 37:569–574.

Zeikus JG, Sawson MA, Thompson TE, Ingvorsen K, Hatchikian EC. 1983. Microbial ecology of volcanic sulfidogenesis: Isolation and characterization of *Thermodesulfobacterium commune* gen. nov. and sp. nov. J Gen Microbiol 129:1159–1169.

Zellner G, Kneifel H, Winter J. 1990. Oxidation of benzaldehydes to benzoic acid derivatives by three *Desulfovibrio* strains. Appl Environ Microbiol 56:2228–2233.

Zillig W, Tu J, Holz I. 1981. Thermoproteales: A third order of thermoacidophilic archaebacteria. Nature (Lond) 293:85–86.

Zillig W, Stetter KO, Prangishvilli D, Schäfer S, Janekovic D, Holz I, Palm P. 1982. Desulfurococcaceae: The second family of the extremely thermophilic, anaerobic, sulfur respiring Thermoproteales. Zentralbl Bakt Hyg I Abt Orig C 3:304–317.

Zillig W, Gierl A, Schreiber G, Wunderl S, Janekovic D, Stetter KO, Klenk HP. 1983. The archaebacterium *Thermofilum pendens*, a novel genus of the thermophilic, anaerobic sulfur respiring Thermoproteales. System Appl Microbiol 4:79–87.

Zimmermann P, Laska S, Kletzin A. 1999. Two modes of sulfite oxidation in the extremely thermophilic and acidophilic archaeon *Acidianus ambivalens*. Arch Microbiol 172:76–82.

19

Biogenesis and Biodegradation of Sulfide Minerals at the Earth's Surface*

19.1 INTRODUCTION

Sulfate-reducing bacteria play an important role in some sedimentary environments in the formation of certain sulfide minerals, especially iron pyrite. Other microbes play an even more pervasive role in the oxidation of a wide range of metal sulfides regardless of the mode of origin of these minerals. The oxidative microbial activity is being industrially exploited in the extraction of metals from some metal sulfide ores. Currently, the bioextractable sulfidic ores of commercial interest include those of copper, nickel, cobalt, and uranium. Although gold in sulfidic ores is not commercially bioextracted, microbial pretreatment (*biobeneficiation*) of such gold ores to remove interfering pyrite and arsenopyrite impurities is now being practiced. The pyrites in these ores encapsulate the gold, making it inaccessible to a chemical extractant such as aqueous cyanide. In the case of cyanide, the pyrites may also cause excessive consumption of the

*In this chapter, the old nomenclature of the Thiobacilli is used to minimize confusion when referring to the scientific literature prior to the year 2000. Based on 16S rRNA gene sequence comparison among the thiobacilli, Kelly and Wood (2000) created the following new generic names for the acidophiles among them: *Thiobacillus thiooxidans, T. ferrooxidans*, and *T. caldus* have been reassigned to the genus *Acidithiobacillus*; *T. acidophilus* has been renamed *Acidiphilium acidophilum*.

TABLE 19.1 Metal Sulfides of Geomicrobial Interest

Mineral or synthetic compound	Formula	Reference
Antimony trisulfide	Sb_2S_3	Silver and Torma (1974); Torma and Gabra (1977)
Argentite	Ag_2S	Baas Becking and Moore (1961)
Arsenopyrite	FeAsS	Ehrlich (1964)
Bornite	Cu_5FeS_4	Cuthbert (1962); Bryner et al. (1954)
Chalcocite	Cu_2S	Bryner et al. (1954); Ivanov (1962); Razzell and Trussell (1963); Sutton and Corrick (1963, 1964); Fox (1967); Nielsen and Beck (1972)
Chalcopyrite	$CuFeS_2$	Bryner and Anderson (1957)
Cobalt sulfide	CoS	Torma (1971)
Covellite	CuS	Bryner et al. (1954); Razzell and Trussell (1963)
Digenite	Cu_9S_5	Baas Becking and Moore (1961); Nielsen and Beck (1972)
Enargite	$3Cu_2S \cdot As_2S_5$	Ehrlich (1964)
Galena	PbS	Silver and Torma (1974)
Gallium sulfide	Ga_2S_3	Torma (1978)
Marcasite, pyrite	FeS_2	Leathen et al. (1953); Silverman et al. (1961)
Millerite	NiS	Razzell and Trussell (1963)
Molybdenite	MoS_2	Bryner and Anderson (1957); Bryner and Jameson (1958); Brierley and Murr (1973)
Orpiment	As_2S_3	Ehrlich (1963a)
Nickel sulfide	NiS	Torma (1971)
Pyrrhotite	Fe_4S_5	Freke and Tate (1961)
Sphalerite	ZnS	Ivanov et al. (1961); Ivanov (1962); Malouf and Prater (1961)
Tetrahedrite	$Cu_8Sb_2S_7$	Bryner et al. (1954)

extractant. A great potential exists for bioextraction of a variety of other metal sulfide ores on an industrial scale.

In the metals industry, a more widely used term for metal bioextraction from ores is **bioleaching**. In this chapter, we examine ore biogenesis and biomobilization, including bioleaching, in some detail. Table 19.1 lists metal sulfide minerals of geomicrobial interest.

19.2 NATURAL ORIGINS OF METAL SULFIDES

Hydrothermal Origin (Abiotic)

Most metal sulfides, including those of commercial interest, are of igneous origin. Current theory explaining their formation invokes plate tectonics, which has

played and is playing a central role in their formation. Terrestrial deposits of **porphyry copper ore** (small crystals of copper sulfides richly dispersed in host rock) are thought to have originated as a result of subduction of oceanic crust that had become somewhat enriched in copper by hydrothermal activity at mid-ocean spreading centers. Subsequent formation of terrestrial deposits of porphyry sulfide ores from subducted oceanic crust is thought to have involved the following successive steps: (1) remelting of the subducted oceanic crust, (2) rising of the resultant magma, (3) release of water with fracturing of incipient rock and the formation of hydrothermal solution containing hydrogen sulfide during progressive partial cooling of the magma, and finally (4) re-formation of copper and other metal sulfides by crystallization of the cooling magma and/or from reaction of H_2S in the hydrothermal solution with metal constituents in the cooled magma in the fractured rock (see Strahler, 1977; Bonatti, 1978; Tittley, 1981).

The enrichment of the surficial deposits of metal sulfide in and on the oceanic crust has occurred and is occurring in hydrothermally active regions at seafloor spreading centers (mid-ocean ridges) at depths of 2500–2600 m. Examples of such sites are the eastern Pacific Ocean at the Galapagos Rift and the East Pacific Rise (Ballard and Grassle, 1979; Corliss et al., 1979) and the Atlantic Ocean on the Mid-Atlantic Ridge (e.g., Klinkhammer et al., 1985). Metal sulfide deposits are evident on the seafloor where some hydrothermal vents ("black smokers"; see Chaps. 2 and 16) discharge brine solution that has a temperature near 350°C and is metal-laden and charged with H_2S. Metal sulfides such as chalcopyrite ($CuFeS_2$) and sphalerite (ZnS) precipitate around the mouth of these vents as the brine meets cold seawater and are often deposited in the form of hollow tubes (chimneys). The hydrothermal solution discharged by these vents originated from seawater that penetrated into porous volcanic rock (basalt) at the mid-ocean spreading centers to depths as great as 10 km below the seafloor (Bonatti, 1978). As this water penetrated ever deeper into the rock, it absorbed heat diffusing away from underlying magma chambers and was subjected to increasing hydrostatic pressure. This caused the seawater to react with the basalt and pick up various metal species and hydrogen sulfide. The reactions responsible for these water modifications include, among others, the interaction of magnesium in the seawater with the rock to form new minerals with an accompanying release of acid (H^+) (Seyfried and Mottl, 1982). The acid leaches metals from the basalt (Edmond et al., 1982; Marchig and Grundlach, 1982). H_2S is formed by reduction of the sulfate in seawater and sulfur in the basalt by ferrous iron released from the basalt (e.g., Shanks et al., 1981; Mottl et al., 1979; Styrt et al., 1981). As long as the hydrothermal solution is subjected to high temperature and pressure in the basalt, metal sulfides are prevented from precipitating.

A quantitatively more significant deposition of metal sulfides occurs within the upper oceanic crust associated with white smokers. Here hot, metal-charged

hydrothermal brine rising from the lower crust meets and mixes with cold seawater that penetrated the upper crust. The mixing of the two solutions in the upper crust results in partial cooling of the solution and consequent precipitation of metal sulfides in the upper crust. This is in contrast to the precipitation of metal sulfides associated with black smokers, which occurs external to the crust around the mouth of the vents and becomes deposited mostly in the walls of vent chimneys. The brine emerging from the vents of white smokers is depleted in some base metals but still contains major quantities of iron, manganese, and hydrogen sulfide. It is much cooler than the hydrothermal solution issuing from the vents of black smokers. Figure 16.17 shows diagrammatically the origin of the hydrothermal solution and metal sulfides associated with black and white smokers at mid-ocean spreading centers.

Sedimentary Metal Sulfides of Biogenic Origin

Among sedimentary metal sulfides of biogenic origin, iron sulfides are the most common. They are usually associated with reducing zones in sedimentary deposits in estuarine environments, which have a plentiful supply of sulfate. The presence of sulfate is important, because the formation of these metal sulfides is usually the result of an interaction of iron compounds with H_2S that originated from bacterial reduction of the sulfate at these sites. The interaction of the H_2S with the iron compounds leads to the formation of iron pyrite (FeS_2). Whether amorphous sulfide (FeS), mackinawite (FeS), and greigite (Fe_3S_4) are intermediates in the formation of the pyrite depends on prevailing environmental conditions (Schoonen and Barnes, 1991a, 1991b; Luther, 1991). In at least one salt marsh (Great Sippewissett Marsh, Massachusetts) where pyrite forms, the pore waters were found to be undersaturated with respect to these compounds (Jørgensen, 1977; Fenchel and Blackburn, 1979; Howarth, 1979; Berner, 1984; Giblin and Howarth, 1984; Howarth and Merkel, 1984). Rapid and extensive microbial pyrite formation has been observed in salt marsh peat on Cape Cod (Massachusetts) (Howarth, 1979). Pyrite formation from biogenic H_2S has also been noted in organic-rich sediments at the Peru Margin of the Pacific Ocean (Mossmann et al., 1991), in Long Island Sound off the Atlantic coast of Connecticut and New York (e.g., Westrich and Berner, 1984), along the Danish coast (Thode-Anderson and Jørgensen, 1989), and in two seepage lakes, Gerritsfles and Kliplo, and two moorland ponds in the Netherlands (Marnette et al., 1993).

In many sedimentary environments, pyrite does not represent a permanent sink for iron because the pyrite may be subject to seasonal reoxidation as conditions in the environment change from reducing to oxidizing (Luther et al., 1982; Giblin and Howarth, 1984; King et al., 1985; Giblin, 1988). Active growth of marsh grass may draw oxygen into the sediment by evapotranspiration (Giblin,

1988). Of all the biogenic sulfide formed in these environments, only a portion is consumed in the formation of pyrite and other metal sulfides. The rest is reoxidized as it enters the oxidizing zones (Jørgensen, 1977). This oxidation may be biological or abiological (Fenchel and Blackburn, 1979).

Nonferrous sulfide deposits of sedimentary origin, especially biogenic ones, appear to be relatively rare. They are generally thought to have formed syngenetically. The metals in question were precipitated by hydrogen sulfide of hydrothermal origin (abiotic formation) or of microbial origin and then buried in contemporaneously formed sediment. The limiting conditions for sedimentary sulfide formation by bacteria as calculated by Rickard (1973) require a minimum of 0.1% carbon (dry weight) and an enriched source of metals such as a hydrothermal solution if more than 1% metal is to be deposited. More recent studies of microbial sulfate reduction revealed, however, that a significant amount of reducing power for sulfate reduction can be furnished by hydrogen (H_2), which would lower the requirement for organic carbon correspondingly (Nedwell and Banat, 1981) (see also Chap. 18, Sec. 18.9).

Examples of nonferrous sedimentary sulfide deposits, all but one of which may have been biogenically formed, include the Permian Kupferschiefer of Mansfeld in Germany (Love, 1962; Stanton, 1972, p. 1139), Black Sea sediments (Bonatti, 1972, p. 51), the Roan Antelope Deposit in Zambia and Katanga (Africa) (Cuthbert, 1962; Stanton, 1972, p. 1139), the Zechstein Deposit in southwestern Poland (Serkies et al., 1967), the deposits in Pernatty Lagoon (Australia) (Lambert et al., 1971), and the Pine Point Pb-Zn property in Northwest Territories, Canada (Powell and MacQueen, 1984). $\delta^{34}S$ analyses of the metal sulfides in the last example suggest an abiotic origin, sulfide having been formed by a reaction between bitumen and sulfate at elevated temperature and pressure.

As an example of ongoing nonferrous sulfide biodeposition, the following observation at the Piquette Pb-Zn deposit in Tennyson, Wisconsin, must be cited. At this site, investigators examined a flooded tunnel in carbonate rock and found the presence of biofilms in which aerotolerant members of sulfate-reducing bacteria of the family Desulfobacteriaceae were precipitating sphalerite (ZnS) at a pH between ~7.2 and 8.6. The sphalerite accumulated in the biofilm in aggregates of particles that had a diameter of 2–5 nm (Labrenz et al., 2000).

Although most instances of metal sulfide biogenesis in nature are associated with bacterial sulfate reduction, at least one case of biogenesis of galena has been attributed to the aerobic mineralization of organic sulfur compounds by *Sarcina flava* Bary (Dévigne, 1968a, 1968b, 1973). The *Sarcina* was isolated from earthy concretions between crystals of galena in an accumulation in a karstic pocket located in the lead-zinc deposit of Djebel Azered, Tunisia. In laboratory experiments, the organism was shown to produce PbS from Pb^{2+} bound to sulfhydryl groups of amino acids in peptone.

19.3 PRINCIPLES OF METAL SULFIDE FORMATION

Metal sulfides in nature result from an interaction between an appropriate metal ion and biogenically or abiotically formed sulfide ion:

$$M^{2+} + S^{2-} \rightarrow MS \tag{19.1}$$

The source of the sulfide in this reaction is what determines whether a metal sulfide is considered to be of biogenic or abiotic origin. In the case of biogenic sulfide, it does not matter whether the sulfide resulted from bacterial sulfate reduction (see Chap. 18) or from bacterial mineralization of organic sulfur-containing compounds (Dévigne, 1968a, 1968b, 1973). Because of their relative insolubility, the metal sulfides form readily at ambient temperatures and pressures. Table 19.2 lists solubility products for some common simple sulfide compounds.

The following calculations will show that relatively low concentrations of metal ions, typical in some lakes, are needed to form metal sulfides by reacting with H_2S. The ionic activities in these calculations are taken as approximately equal to concentration because of the low concentrations involved. The following examines the case of amorphous iron sulfide (FeS) formation.

The ionization constant for FeS is

$$[Fe^{2+}][S^{2-}] = 10^{-19} \tag{19.2}$$

The ionization constant for H_2S is

$$[S^{2-}] = 10^{-21.96}\,\frac{[H_2S]}{[H^+]^2} \tag{19.3}$$

This relationship is derived from the constant for the dissociation of H_2S into HS^- and H^+,

$$\frac{[HS^-]\,[H^+]}{[H_2S]} = 10^{-6.96} \tag{19.4}$$

TABLE 19.2 Solubility Products for Some Metal Sulfides

CdS	1.4×10^{-28}	FeS	1×10^{-19}	NiS	3×10^{-21}
Bi_2S_3	1.6×10^{-72}	PbS	3.4×10^{-28}	Ag_2S	1×10^{-51}
CoS	7×10^{-23}	MnS	5.6×10^{-16}	SnS	8×10^{-29}
Cu_2S	2.5×10^{-50}	Hg_2S	1×10^{-45}	ZnS	1.2×10^{-23}
CuS	8.5×10^{-45}	HgS	3×10^{-53}	H_2S	1.1×10^{-7}
				Hs^-	1×10^{-15}

Source: Latimer and Hildebrand, 1942; Weast and Astle, 1982.

and the constant for the dissociation of HS^- into S^{2-} and H^+,

$$\frac{[S^{2-}]\,[H^+]}{[HS^-]} = 10^{-15} \tag{19.5}$$

Substituting Eq. (19.3) into Eq. (19.2), the following relationship is obtained:

$$[Fe^{2+}] = \frac{[H^+]^2}{[H_2S]} \times \frac{10^{-19}}{10^{-21.96}} = \frac{[H^+]^2}{[H_2S]} \times 10^{2.96} \tag{19.6}$$

Assuming that the bottom water of a lake contains about 34 mg of $H_2S\,L^{-1}$ (10^{-3} M) at pH 7, about 5.08 μg $Fe^{2+}\,L^{-1}$ ($10^{-7.04}$ M) will be precipitated as FeS by 3.4 mg of hydrogen sulfide per liter (10^{-4} M). The unused H_2S will ensure reducing conditions, which will keep the iron in the ferrous state. Because ferrous sulfide is one of the more soluble sulfides, metals whose sulfides have even smaller solubility products will require even less sulfide for precipitation. In the excess of sulfide, the FeS would probably be transformed into FeS_2, which is more stable than FeS.

19.4 LABORATORY EVIDENCE IN SUPPORT OF BIOGENESIS OF METAL SULFIDES

Batch Cultures

Metal sulfides have been generated in laboratory experiments using H_2S from bacterial sulfate reduction. Miller (1949, 1950) reported that sulfides of Sb, Bi, Co, Cd, Fe, Pb, Ni, and Zn were formed in a lactate-containing broth culture of *Desulfovibrio desulfuricans* to which insoluble salts of selected metals had been added. For instance, he found that bismuth sulfide was formed on addition of $(BiO_2)_2CO_3 \cdot H_2O$, cobalt sulfide on addition of $2CoCO_3 \cdot 3Co(OH)_2$, lead sulfide on addition of $2PbCO_3 \cdot Pb(OH)_2$ or $PbSO_4$, nickel sulfide on addition of $NiCO_3$ or $Ni(OH)_2$, and zinc sulfide on addition of $2ZnCO_3 \cdot 3Zn(OH)_3$ as starting compounds. Insoluble metal salts were added as starting compounds to minimize metal toxicity for *D. desulfuricans*. Metal ion toxicity depends in part on the solubility of the metal compound from which the ion derives. Obviously, for a metal sulfide to be formed from another metal compound that is relatively insoluble, the metal sulfide must be even more insoluble than the source compound of the metal. Miller was not able to demonstrate copper sulfide formation from malachite [$CuCO_3 \cdot Cu(OH)_2$], probably because malachite was too insoluble relative to copper sulfides in his medium. Miller (1949) also showed that with addition of Cd or Zn ions to the culture medium, the yield of total sulfide produced by the bacteria in batch culture was greater than in the absence

of the added metal ions. This was because uncombined sulfide at high enough concentration itself becomes toxic to sulfate reducers.

Baas Becking and Moore (1961) also undertook a study of biogenesis of sulfide minerals. Like Miller, they worked with batch cultures of sulfate-reducing bacteria. The bacteria they employed were *Desulfovibrio desulfuricans* and *Desulfotomaculum* sp. (which they called *Clostridium desulfuricans*). They grew them in lactate or acetate medium containing steel wool. The steel wool in the medium was meant to serve as a source of hydrogen for the bacterial reduction of sulfate. The hydrogen resulted from corrosion of the steel wool by the reaction,

$$Fe^0 + 2H_2O \rightarrow H_2 + Fe(OH)_2 \tag{19.7}$$

The H_2 was then used by the sulfate reducers in the formation of hydrogen sulfide,

$$4H_2 + SO_4{}^{2-} + 2H^+ \rightarrow H_2S + 4H_2O \tag{19.8}$$

The media were saline to simulate marine (near-shore and estuarine) conditions under which the investigators thought the reactions are likely to occur in nature. They formed ferrous sulfide from strengite ($FePO_4$) and from hematite (Fe_2O_3). They also formed covellite (CuS) from malachite [$CuCO_3 \cdot Cu(OH)_2$]; argentite (Ag_2S) from silver chloride (Ag_2Cl_2) and from silver carbonate ($AgCO_3$); galena (PbS) from lead carbonate ($PbCO_3$) and from lead hydroxycarbonate [$PbCO_3 \cdot Pb(OH)_2$]; and sphalerite (ZnS) from smithsonite ($ZnCO_3$). All mineral products were identified by X-ray powder diffraction studies. Baas Becking and Moore (1961) were unable to form cinnabar from mercuric carbonate ($HgCO_3$), probably owing to the toxicity of the Hg^{2+} ion. They were also unable to form alabandite (MnS) from rhodochrosite ($MnCO_3$), or bornite (Cu_5FeS_4) or chalcopyrite ($CuFeS_2$) from a mixture of cuprous oxide (Cu_2O) or malachite plus hematite and lepidochrosite. They succeeded in forming covellite from malachite where Miller (1950) failed, probably because they performed their experiment in a saline medium (3% NaCl) in which Cl^- could complex Cu^{2+}, thereby increasing the solubility of Cu^{2+}. The starting materials that were the source of metal were all relatively insoluble, as in Miller's experiments. Baas Becking and Moore found that in the formation of covellite and argentite, native copper and silver were respective intermediates that disappeared with continued bacterial H_2S production.

Leleu et al. (1975) synthesized ZnS by passing H_2S produced by unnamed strains of sulfate-reducing bacteria through a solution of $ZnSO_4$. In one experiment, biogenic H_2S formation and zinc sulfide precipitation by the biogenic H_2S occurred in separate vessels. In a second experiment, biogenesis of H_2S and precipitation of ZnS occurred in the same vessel at an initial $ZnSO_4$ concentration

in the culture medium of 10^{-2} M. The ZnS formed under either experimental condition was identified as a sphalerite–wurtzite mixture by powder X-ray diffraction diagrams. The presence of Zn directly in the culture medium caused a lag in H_2S production, which was not observed when H_2S was generated in a separate vessel.

Column Experiment: A Model for Biogenesis of Sedimentary Metal Sulfides

The relatively high toxicity of many of the heavy metals for sulfate-reducing bacteria has been used as an argument that these organisms could not have been responsible for metal sulfide precipitation in nature (e.g., Davidson, 1962a, 1962b). However, in a sedimentary environment, metal ions will be mostly adsorbed to sediment particles such as clays or complexed by organic matter (Hallberg, 1978), which lessens their toxicity. Such adsorbed or complexed ions are still capable of reacting with sulfide and precipitating as metal sulfides, as was shown experimentally by Temple and LeRoux (1964). They constructed a column in which a clay or ferric hydroxide slurry carrying adsorbed Cu^{2+}, Pb^{2+}, and Zn^{2+} ions was separated by an agar plug from an underlying liquid culture of sulfate reducers actively generating hydrogen sulfide in saline medium. They also tested clay that was carrying Fe^{3+} in this setup. They found that, in time, bands of precipitate formed in the agar plug separating a slurry of metal-carrying adsorbent from the culture of sulfate-reducing bacteria (Fig. 19.1). The bands formed as the upward-diffusing sulfide ion species and the downward-diffusing, desorbed metal ion species encountered each other in the agar. Differential desorption of metal ions from the adsorbent and the differential diffusion in the agar accounted for the discrete banding by the various sulfides. These results demonstrate that biogenesis of relatively large amounts of sulfides in a sedimentary environment is possible, even in the presence of relatively large amounts of metal ions. The main requirement is that the metal ions are in a nontoxic form (e.g., adsorbed or complexed) or combined in the form of insoluble mineral oxide, carbonate, or sulfate. As Temple (1964) pointed out, syngenetic microbial production of metal sulfide in nature is possible. Restrictions on the process, according to him, are not metal toxicity but free movement of the bacterially generated sulfide and a need for metal-enriched zones in the sedimentary environment. On a biochemical basis, Temple suspected that microbial sulfate reduction evolved in the Precambrian. Subsequent stable sulfur isotope analyses of samples representing the early Precambrian in South Africa indicated that extensive biogenic sulfate reduction occurred at least 2350 million years ago (Cameron, 1982).

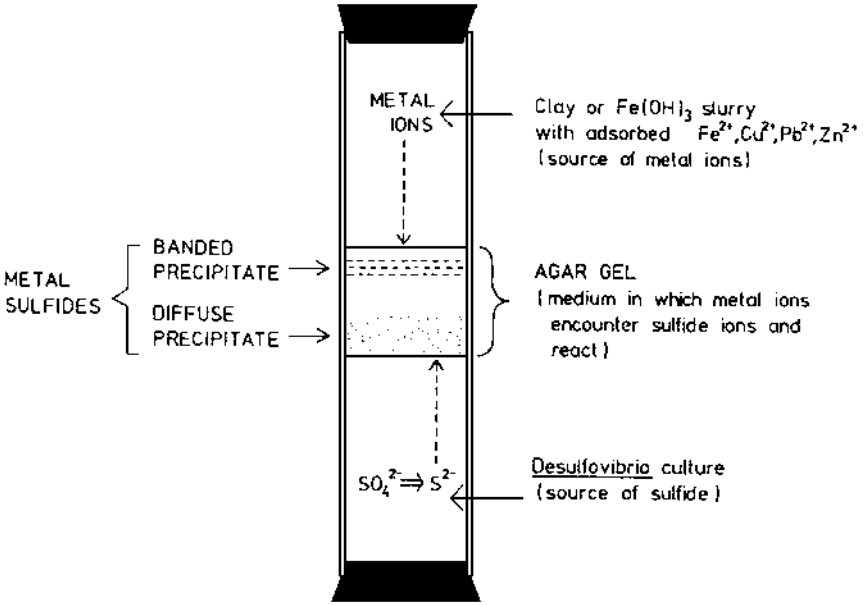


FIG. 19.1 Temple and LeRoux column showing how sulfate reducers can precipitate metal sulfides by reaction with biogenic sulfides with metal ions. The absorbents, clay or $Fe(OH)_3$ slurry, control the concentration of metal ions in solution, and the agar plug prevents physical contact of the sulfate reducers with metal ions. In nature, sediments can act as adsorbents of metal ions. They hold the metal ion concentration in the interstitial water at such a level that sulfate reducers are not poisoned.

19.5 BIO-OXIDATION OF METAL SULFIDES

Regardless of whether they are of abiogenic or biogenic origin, metal sulfides in nature may be subject to microbial oxidation. This may take the form of **direct** or **indirect** interaction (Silverman and Ehrlich, 1964). In *direct interaction*, the microbes oxidize a metal sulfide in direct contact with the mineral surface. In *indirect interaction*, the microbes usually generate an oxidant (commonly ferric iron from ferrous iron) in the bulk phase. The oxidant then attacks the metal sulfide. In most instances, the metal is solubilized as a metal ion by either mode of oxidation. The bio-oxidation of galena (PbS) is an exception because the mobilized metal reacts with sulfate ion, which is generated during the oxidation and which is also present in the bulk phase, to form insoluble lead sulfate ($PbSO_4$). Some microbes can mobilize metals in metal sulfides in an indirect mode by generating ligands, which may also be acids. These mobilize the metals by complexing them and thereby keeping them in solution.

Organisms Involved in Bio-oxidation of Metal Sulfides

A number of different acidophilic, iron-oxidizing bacteria have been detected at sites where metal sulfide oxidation is occurring (Norris, 1990; Rawlings, 1997b). The most important of these have been identified as the mesophiles *Thiobacillus ferrooxidans, Leptospirillum ferrooxidans, Ferroplasma acidiphilum*, and *Ferroplasma acidarmanus*; the moderate thermophiles *Sulfobacillus thermosulfidooxidans* and *Acidimicrobium ferrooxidans*; and the extreme thermophiles *Sulfolobus* spp. and *Acidianus brierleyi* (formerly *Sulfolobus brierleyi*). All are autotrophs, and all but *F. acidarmanus* grow best in a pH range of about 1.5–2.5. *F. acidarmanus*, a very recent discovery, grows at a pH as low as 0 (optimum pH 1.2) at a temperature of ~40°C. It was isolated from pyrite surfaces of the ore body at Iron Mountain, California, and is a cell-wall-lacking, iron-oxidizing autotroph (Edwards et al., 2000). *F. acidiphilum*, also discovered recently and a close relative of *F. acidarmanus*, was isolated from a bioleaching pilot plant (Golyshina et al., 2000). It grows in a pH range of 1.3–2.2 (optimum pH 1.7) in a temperature range of 15–45°C. *T. ferrooxidans*, *L. ferrooxidans*, *S. thermosulfidooxidans*, and *A. ferrooxidans* are members of the domain Bacteria (Norris, 1997). *Sulfolobus* spp., *Acidianus brierleyi*, *Ferroplasma acidarmanus*, and *F. acidiphilum* are members of the domain Archaea. Whereas *Leptospirillum ferrooxidans* and *Acidimicrobium ferrooxidans* oxidize Fe^{2+} and pyrite, they do not oxidize reduced sulfur as *T. ferrooxidans* and *S. thermosulfidooxidans* do. This seems to suggest that *L. ferrooxidans* and *A. ferrooxidans* can promote metal sulfide oxidation only by generating Fe^{3+} from dissolved Fe^{2+}, which then oxidizes metal sulfide by abiotic means. However, because of a structural feature possessed by both *T. ferrooxidans* and *L. ferrooxidans*, both organisms may also be able to oxidize metal sulfides by attacking them directly. The common structural feature is exopolymer (EPS) secreted by the cells that contains bound iron (Gehrke et al., 1995, 1998; Sand et al., 1997). The exopolymer enables attachment to sulfide mineral surfaces. In addition, as will be explained below, the iron in the EPS may serve as an electron shuttle for conveying electrons in the oxidation of metal sulfides to the electron transport system in the plasma membrane of the cells. It remains to be determined if *A. ferrooxidans* forms EPS with bound iron.

Whereas *T. ferrooxidans*, *Sulfolobus*, and *Acidianus brierleyi* are autotrophs, growth of the latter two organisms is stimulated by a trace of yeast extract in laboratory culture. In the absence of dissolved ferrous iron or reduced forms of sulfur in the medium, all three organisms can use appropriate metal sulfides as energy sources. Depending on the oxidation state of the metal moiety in the metal sulfide, both it and the sulfide may serve as energy sources. For example, in the oxidation of chalcocite (Cu_2S), *T. ferrooxidans* can use the energy from Cu(I)

oxidation for CO_2 fixation (Nielsen and Beck, 1972) (see also further discussion in the next section). Cell extracts from *T. ferrooxidans* have been prepared that catalyze the oxidation of a cuprous copper in Cu_2S but not of elemental sulfur (Imai et al., 1973). The oxidation is not inhibited by quinacrine (atebrine). It needs the addition of a trace of iron for proper activity. The effect of traces of iron on metal sulfide oxidation had been previously noted in experiments in which the addition of 9 mg of ferrous iron per liter of medium stimulated metal sulfide oxidation by whole cells of *T. ferrooxidans* (Ehrlich and Fox, 1967).

Thiobacillus ferrooxidans can use NH_4^+ and some amino acids as nitrogen sources (see Sugio et al., 1987) (see also Chap. 15). At least some strains are able to fix nitrogen (Mackintosh, 1978; Stevens et al., 1986).

T. ferrooxidans is very versatile in attacking metal sulfides. It has been reported to oxidize arsenopyrite (FeS_2FeAs_2 or FeAsS), bornite (Cu_5FeS_4), chalcocite (Cu_2S), chalcopyrite ($CuFeS_2$), covellite (CuS), enargite ($3Cu_2S{\cdot}As_2S_5$), galena (PbS), millerite (NiS), orpiment (As_2S_3), pyrite (FeS_2), marcasite (FeS_2), sphalerite (ZnS), stibnite (Sb_2S_3), and tetrahedrite ($Cu_8Sb_2S_7$) (see Silverman and Ehrlich, 1964). In addition, the oxidation of gallium sulfide, synthetic preparations of CoS, NiS, and ZnS, and pyrrhotite by *T. ferrooxidans* has been reported (Torma, 1971, 1978; Pinka, 1991; Bhatti et al., 1993). The mode of attack of any of these minerals may be direct, indirect, or both.

Although not as exhaustively tested as *T. ferroxidans, Acidianus brierleyi* and *Sulfolobus* sp. can also oxidize a variety of metal oxides including pyrite, arsenopyrite, chalcopyrite, NiS, and probably CoS (Brierley, 1978a, 1978b, 1982; Brierley and Murr, 1973; Dew et al., 1999). Unlike *T. ferrooxidans*, *A. brierleyi* can oxidize molybdenite in the absence of added iron (Brierley and Murr, 1973) because molybdate iron is less toxic to it than it is to *T. ferrooxidans* (Tuovinen et al., 1971).

Direct Oxidation

According to the concept of direct oxidation of susceptible metal sulfides as defined by Silverman and Ehrlich (1964), the crystal lattice of such sulfides is attacked through enzymatic oxidation. To accomplish this, the microbes have to be in intimate contact with the mineral they attack. Evidence for rapid attachment of *T. ferrooxidans* to mineral surfaces of chalcopyrite particles ($CuFeS_2$) has been presented by McGoran et al. (1969) and Shrihari et al. (1991); to covellite particles by Pogliani et al. (1990); to galena crystals by Tributsch (1976); to pyrite crystals by Bennett and Tributsch (1978), Rodriguez-Leiva and Tributsch (1988), Mustin et al. (1992), Murthy and Natarajan (1992), and Edwards et al. (1998); and to pyrite/arsenopyrite-containing auriferous ore by Norman and Snyman (1988).

The mere spontaneous dissociation of a mineral to yield oxidizable ion species in solution that *T. ferrooxidans* can attack is too small in the case of minerals that are very insoluble in acid solution. For example, covellite (CuS), in which the only oxidizable constituent is the sulfide, has a solubility constant of $10^{-44.07}$ (Table 19.2). *Thiobacillus ferrooxidans* is able to oxidize this mineral at pH 2.0 (see later in this section). Simple calculations show that at equilibrium at pH 2 in water, the dissociation of CuS will only generate a concentration of HS^- equal to $10^{-15.53}$ M and a concentration of H_2S equal to $10^{-13.06}$ M. This is insufficient for sulfide oxidation by *T. ferrooxidans* because the most recent K_s value for sulfide oxidase in intact cells of this organism has been reported to be $10^{-5.30}$ M (Pronk et al., 1990). K_s values are a measure of the substrate concentration at which a reaction catalyzed by intact cells is half-maximal (Michaelis–Menten kinetics). (See footnote in Chap. 15, Sec. 15.4. K_s for a cell is equivalent to K_m for an individual enzyme.) Thus the mere dissociation of CuS into Cu^{2+}, HS^-, and H_2S cannot furnish nearly enough sulfide substrate to sustain its oxidation by *T. ferrooxidans* at a reasonable velocity, regardless of whether HS^- or H_2S or both are the actual substrate for sulfide oxidase. Because *T. ferrooxidans* can oxidize covellite in the absence of added iron, it must be in direct contact with a mineral to attack it. The need for direct contact in covellite oxidation by *T. ferrooxidans* in the absence of added Fe^{2+} was demonstrated experimentally by Pogliani et al. (1990). By contrast, *T. thiooxidans* was shown by Donati et al. (1995) to promote covellite oxidation only with the addition to the medium of Fe(III) or by Fe(II) autoxidized to Fe(III). The oxidation of covellite by Fe(III) generated S^0 (and possibly small amounts of other partially reduced and dissolved sulfur species) as first described by Sullivan (1930),

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + S^0 + 2Fe^{2+} \tag{19.9}$$

As pointed out in Chap. 18, *T. thiooxidans* cannot oxidize Fe^{2+}.

On the other hand, if we consider a more soluble sulfide mineral such as ZnS, which has a solubility constant of $10^{-22.9}$, calculations similar to those for CuS show that at pH 2.0, ZnS dissociation will yield $10^{-4.95}$ M HS^- and $10^{-1.47}$ M H_2S (Table 19.2). These concentrations of HS^- and H_2S are more than sufficient to satisfy the K_s of $10^{-5.30}$ for sulfide oxidase in *T. ferrooxidans* and to permit its growth without direct attack of ZnS at the mineral surface. Indeed, it has been shown that *T. thiooxidans*, which is unable to oxidize Fe^{2+}, will readily promote the dissolution of ZnS at pH 2.0 (Pistorio et al., 1994). The relative solubility of PbS in acid solution also explains why Garcia et al. (1995) found that *T. thiooxidans* promoted the dissolution of PbS (galena). The solubility constant of this metal sulfide is $10^{-27.5}$, a little smaller than that of ZnS ($10^{-22.9}$) but significantly larger than that of CuS ($10^{-44.07}$). At pH 2, PbS dissociates to yield $10^{-7.25}$ M HS^- and $10^{-4.77}$ M H_2S (Table 19.2).

The exact nature of the interaction between a sulfide mineral surface and the *T. ferrooxidans* cell surface, on which the enzyme-catalyzed oxidation of the mineral depends, is still unknown. Iron bound in the cell envelope of *T. ferrooxidans* has been postulated to serve as an electron shuttle that conveys electrons from an external electron donor across the outer membrane to the electron carriers in the periplasm of the cell (see Ingledew, 1986; Ehrlich, 2000). Alternatively, Tributsch (1999) views the ferric iron bound in the exopolymer at the cell surface of *T. ferrooxidans* in contact with a mineral surface as generating elemental sulfur according to reaction (19.9). This sulfur is then oxidized by *T. ferrooxidans* by a known reaction (see Chap. 18). However, this assumes that EPS-bound iron is as strong an oxidant as iron in the bulk phase.

Precedents for electron transfer between an outer cell surface and a mineral surface with which it is in contact exist among bacteria that are involved in the reduction of ferric oxide and MnO_2 (see Chaps. 15 and 16). *Shewanella putrefaciens* introduces c-type cytochromes (OmcA, OmcB) into its outer membrane when it is growing anaerobically. These cytochromes play a role in the reduction of MnO_2, which is extremely insoluble at the circumneutral pH at which the reduction occurs (Myers and Myers, 1997, 1998, 2001; see also Lower et al., 2001, with regard to goethite attack). Similar observations have been made with the obligate anaerobe *Geobacter sulfurreducens*, which can reduce insoluble ferric oxides and MnO_2 (Lovley, 2000). In Lovley's structural model of the enzyme system, based on the location of the components in the cell envelope, a 41 kDa cytochrome, which is involved in the transfer of electrons from the cell surface to Fe(III) or Mn(IV) oxide, is linked to an 89 kDa cytochrome in the plasma membrane by a 9 kDa cytochrome in the periplasm (Lovley, 2000). The 89 kDa cytochrome in the plasma membrane is reduced by NADH via an NADH dehydrogenase complex according to Lovley. Ehrlich (1993a, 1993b) proposed a different mechanism for transfer of electrons from the surface of a marine bacterium (strain BIII 88) to the surface of MnO_2 with which it is in contact. In his model, Mn^{2+} bound in the cell envelope undergoes a disproportionation reaction with Mn(IV) at the surface of MnO_2 particles, forming Mn(III). It is this Mn(III) that is reduced to Mn(II) by the cell with a suitable electron donor, e.g., acetate (Ehrlich 1993a, 1993b) (see also Chap. 16). The reduction in this case has been shown to occur aerobically as well as anaerobically. This model resembles the one proposed by Ingledew (1986) (see also Ehrlich et al., 1991) for Fe^{2+} and metal sulfide oxidation. In this model, polynuclear iron bound in the outer membrane of *T. ferrooxidans* serves to convey electrons from a donor (e.g., Fe^{2+}) to the electron transport components in the periplasm (c-type cytochrome and rusticyanin) by being reversibly reduced and oxidized. The iron bound in the exopolymeric substance (EPS) around the cells of *T. ferrooxidans* described by Gehrke et al. (1995, 1998) and Sand et al. (1997) should be included in the polynuclear iron. The role that Sand et al. (1997) assigned to the EPS-bound iron

is that of mediating (or at least initiating) attack of the sulfur moiety in pyrite. They view bulk-phase iron as inducing EPS formation and enabling attachment to pyrite. In the model described in the present discussion, the polynuclear iron acts as an *electron shuttle* and not as a chemical reactant. The standard reduction potential for the Fe^{3+}/Fe^{2+} couple is +777 mV, that of the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ in 0.01 N NaOH is +460 mV (Weast and Astle, 1982), and that for oxidized cytochrome c/reduced cytochrome c is +0.254 mV (Lehninger, 1975, p. 479). Although no reduction potential for EPS-bound iron is available, its value is likely to lie between that for uncomplexed iron and the porphyrin-bound iron in cytochrome. EPS-bound iron would therefore be a significantly weaker oxidant and function better as an electron shuttle.

Bacterial attachment to mineral sulfide surfaces appears not to be random but to occur at specific sites and even specific crystal faces. Some evidence suggests that direct microbial attack is initiated at sites of crystal imperfections. Selective attachment of *T. ferrooxidans* or *Sulfolobus acidocaldarius* to newly exposed pyrite crystals in coal is very rapid, i.e., about 90% complete in 2–5 min (Bagdigian and Myerson, 1986; Chen and Skidmore, 1987, 1988). Although the details of how microbes attack crystal lattices of metal sulfides once they have attached are not yet understood, a model can be formulated. In this model, the bacterial cells act as catalytic conductors in transferring electrons from cathodic areas on crystal surfaces of a metal sulfide via an electron transport system in their cell envelope to oxygen (Fig. 19.2). The electron transport system of the bacteria acts as a catalyst. Indeed, the bacteria can be viewed as cathodic extensions. They benefit from this process by coupling energy conservation (ATP synthesis) to it.

Evidence for enzymatic attack of synthetic covellite (CuS) through measurement of oxygen consumption and Cu^{2+} and SO_4^{2-} ion production by *T. ferrooxidans* in the presence and absence of the enzyme inhibitor trichloroacetate (8 mM) was obtained by Rickard and Vanselow (1978). In the case of CuS, only the sulfide moiety of the mineral is attacked because the metal moiety is already as oxidized as possible. The oxidation of the mineral probably proceeds in two steps (Fox, 1967):

$$CuS + 0.5O_2 + 2H^+ \xrightarrow{\text{bacteria}} Cu^{2+} + S^0 + H_2O \tag{19.10}$$

$$S^0 + 1.5O_2 + H_2O \xrightarrow{\text{bacteria}} H_2SO_4 \tag{19.11}$$

By contrast, *Thiobacillus thioparus* promotes covellite oxidation only after autoxidation of the mineral to $CuSO_4$ and S^0 [similar to reaction (19.10) but without bacterial catalysis] (Rickard and Vanselow, 1978). It is the bacterial catalysis of the oxidation of S^0 to sulfate that helps the reaction by removing a product of the autoxidation of the CuS.

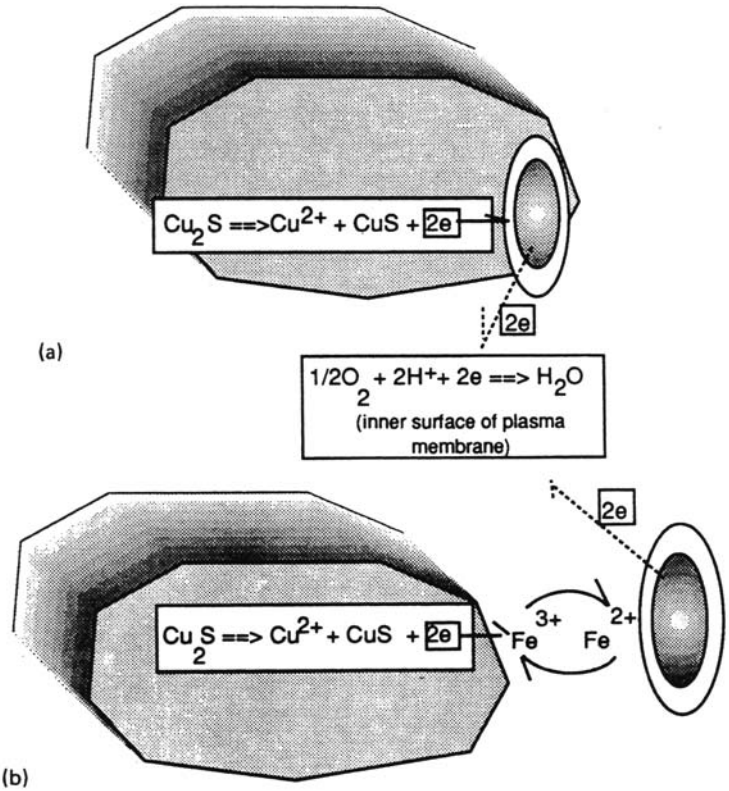


FIG. 19.2 Schematic representation of direct and indirect oxidation of a particle of Cu_2S by *Thiobacillus ferrooxidans*. (a) Direct oxidation. In this model the bacterial cell acts essentially as a conductor of electrons from the crystal lattice of Cu_2S to oxygen while attached to the particle surface. Not shown is the mechanism by which the electrons cross the interface between the particle surface and the cell surface. For possible mechanisms used by *T. ferrooxidans*, see Chapter 15, Figures 15.3a and 15.3b, and discussion in Section 19.5 of this chapter. (b) Indirect oxidation. In this model planktonic (unattached) bacterial cells generate and regenerate the oxidant (Fe^{3+}) in the bulk phase, which acts as a shuttle that carries electrons from the crystal lattice of Cu_2S to the bacterium, which transfers the electron to oxygen.

In some instances, both an oxidizable metal moiety and the sulfide moiety may be attacked by separate enzymes, as for example in the case of chalcopyrite ($CuFeS_2$) (assuming the Fe of chalcopyrite to have an oxidation state of $+2$) (Duncan et al., 1967; Shrihari et al., 1991). Although Duncan and coworkers reported Fe and S to be simultaneously attacked, Shrihari et al. (1991) found that

iron-grown *T. ferrooxidans* oxidized the sulfide sulfur of chalcopyrite by direct attack before oxidizing ferrous iron in solution to ferric iron. When the dissolved ferric iron attained a significant concentration, it promoted chemical oxidation of residual chalcopyrite. The overall reaction by *T. ferrooxidans* may be written as follows:

$$4CuFeS_2 + 17O_2 + 4H^+ \xrightarrow{T.\ ferrooxidans} 4Cu^{2+} + 4Fe^{3+} + 8SO_4{}^{2-} + 2H_2O \quad (19.12)$$

$$4Fe^{3+} + 12H_2O \longrightarrow 4Fe(OH)_3 + 12H^+ \quad (19.13)$$

$$4CuFeS_2 + 17O_2 + 10H_2O \xrightarrow{T.\ ferrooxidans} 4Cu^{2+} + 4Fe(OH)_3 + 8SO_4{}^{2-} + 8H^+ \quad (19.14)$$

Reaction (19.14) is the sum of reactions (19.12) and (19.13).

In other cases of direct attack, the oxidizable metal moiety may be oxidized before the sulfide, as in the case of chalcocite (Cu_2S) oxidation (Fox, 1967; Nielsen and Beck, 1972) (Fig. 19.2):

$$Cu_2S + 0.5O_2 + 2H^+ \xrightarrow{T.\ ferrooxidans} Cu^{2+} + CuS + H_2O \quad (19.15)$$

$$CuS + 0.5O_2 + 2H^+ \xrightarrow{T.\ ferrooxidans} Cu^{2+} + S^0 + H_2O \quad (19.16)$$

$$S^0 + 1.5O_2 + H_2O \xrightarrow{T.\ ferrooxidans} H_2SO_4 \quad (19.17)$$

Digenite (Cu_9S_5) can be an intermediate in the formation of CuS from Cu_2S (Nielsen and Beck, 1972).

Although in the laboratory it is possible to demonstrate exclusive direct leaching by *T. ferrooxidans* of certain nonferrous metal sulfides by using iron-free mineral in an iron-free culture medium, in nature these conditions never occur. This is because nonferrous metal sulfides are always accompanied by pyrites in ore deposits. Thus, in nature, direct and indirect oxidation usually occur together. It is prevailing environmental conditions that determine the extent to which each mode contributes to overall oxidation of a metal sulfide. Pyrite oxidation presents a special problem in applying the concepts of direct and indirect attack in bacterial leaching and is treated in a special section to follow.

Indirect Oxidation

In indirect bio-oxidation of metal sulfides, a major role of the bacteria is the generation of a lixiviant, which chemically oxidizes the sulfide ore. This lixiviant is ferric iron (Fe^{3+}). It is a major **consumable reactant** in the oxidation of the metal sulfide. It may be generated initially from dissolved ferrous iron (Fe^{2+}) at pH values of 3.5–5.0 by *Metallogenium* in a mesophilic temperature range (Walsh

and Mitchell, 1972a). At pH values below 3.5, ferric iron may be generated from Fe^{2+} by bacteria such as *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* Markosyan (see Balashova et al., 1974) in a mesophilic temperature range and by *Sulfolobus* spp., *Acidianus brierleyi, Sulfobacillus thermosulfidooxidans*, and others in a thermophilic temperature range (Brierley, 1978b; Brierley and Brierley, 1973; Brierley and Murr, 1973; Brierley and Lockwood, 1977; Brierley et al., 1978; Balashova et al., 1974; Golovacheva and Karavaiko, 1979; Harrison and Norris, 1985; Pivovarova et al., 1981; Segerer et al., 1986). In addition, ferric iron can be generated from iron pyrites (e.g., FeS_2) by *T. ferrooxidans* and other iron-oxidizing acidophiles, some of which are also capable of direct attack as described previously. In whatever way it is formed, ferric iron in acid solution acts as an oxidant of the metal sulfides (e.g., Sullivan, 1930; Ehrlich and Fox, 1967):

$$MS + 2Fe^{3+} \rightarrow M^{2+} + S^0 + 2Fe^{2+} \tag{19.18}$$

where M may be any metal in an appropriate oxidation state, which does not always have to be divalent. A central role of *T. ferrooxidans* in an indirect leaching process is to regenerate Fe^{3+} from the Fe^{2+} formed in reaction (19.18). It should be noted that in this type of chemical reaction the sulfide of a mineral is mostly oxidized to elemental sulfur (S^0) (pyrite is an exception). Further oxidation to sulfuric acid (H_2SO_4) is very slow but is likely to be greatly accelerated by microorganisms like *T. thiooxidans, T. ferrooxidans, Sulfolobus* spp., and *Acidianus brierleyi* but not *L. ferrooxidans*. Elemental sulfur may form a film on the surface of metal sulfide crystals in chemical oxidation and interfere with the further chemical oxidation of the residual metal sulfide. The chemical oxidation of metal sulfides must occur in acid solution below pH 5.0 to keep enough ferric iron in solution. In nature, the needed acid may be formed chemically through autoxidation of sulfur and other partially reduced forms of sulfur, or more likely biologically through bacterial oxidation of the sulfur. The acid may also form as a result of autoxidation of ferrous iron or pyrite. In ferrous iron oxidation, the acid forms as follows:

$$2Fe^{2+} + 0.5O_2 + 2H^+ \xrightarrow{\text{bacteria}} 2Fe^{3+} + H_2O \tag{19.19}$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \tag{19.20}$$

Because this reaction normally occurs in the presence of sulfate, the ferric hydroxide may convert to the more insoluble jarosite, especially in the presence of *T. ferrooxidans* and probably other acidophilic iron oxidizers (Lazaroff, 1983; Lazaroff et al., 1982, 1985; Carlson et al., 1992):

$$A^+ + 3Fe(OH)_3 + 2{SO_4}^{2-} \rightarrow AFe_3(SO_4)_2(OH)_6 + 3OH^- \tag{19.21}$$

where A^+ may represent Na^+, K^+, NH_4^+, or H_3O^+ (Duncan and Walden, 1972). The formation of jarosite decreases the ratio of protons produced per iron oxidized from 2:1 to 1:1. In pyrite oxidation, the acid forms as a result of reactions (19.22), (19.23), and (19.24) (see below). Reaction (19.22) proceeds by autoxidation if the process is indirect. Like sulfur, jarosite may also form on the surface of metal sulfide crystals and block further oxidation.

Pyrite Oxidation

Pyrite oxidation by *T. ferrooxidans* represents a special case in which direct and indirect oxidation of the mineral cannot be readily separated because ferric iron is always a product. Experimentally, Mustin et al. (1992) recognized four phases in the leaching of pyrite by *T. ferrooxidans* in a stirred reactor. The first phase, which lasted about 5 days, featured a measurable decrease in unattached bacteria. The small amount of dissolved ferric iron added with the inoculum was reduced by reacting with some of the pyrite. The second phase, which also lasted about 5 days, featured the start of pyrite dissolution with oxidation of its iron and sulfur, but with sulfur being preferentially oxidized. Unattached bacteria multiplied exponentially, and the pH began to drop. The third phase, which lasted about 10 days, featured a significant increase in dissolved ferric iron, the ferrous iron concentration remaining low. Both iron and sulfur in the pyrite were being oxidized at high rates. However, the rate of sulfur oxidation decreased with time relative to iron oxidation, the ratio of sulfate to ferric iron becoming stoichiometric by day 18. Unattached bacteria continued to increase exponentially, and the pH continued to drop. The surface of the pyrite crystals began to show evidence of corrosion cracks. In the fourth and last phase, which lasted about 25 days, the dissolved Fe(III)/Fe(II) ratio decreased slightly, iron and sulfur in the pyrite continued to be strongly oxidized, and the unattached bacteria reached a stationary phase. At the same time, the pH continued to drop to 1.3 by the 45th day. The surface of the pyrite particles now showed easily recognizable square or hexagonal corrosion pits. During the entire experiment of pyrite oxidation, Mustin and coworkers followed pH and electrochemical (redox) changes.

Classically, direct bacterial oxidation of iron pyrite (FeS_2) has been summarized by the reaction

$$FeS_2 + 3.5O_2 + H_2O \xrightarrow{\text{attached } T.\ ferrooxidans} Fe^{2+} + 2H^+ + 2SO_4{}^{2-} \tag{19.22}$$

The ferrous iron generated in this reaction is further oxidized by planktonic bacteria (not attached to pyrite surfaces) according to the overall reaction

$$2Fe^{2+} + 0.5O_2 + 2H^+ \xrightarrow{\text{planktonic } T.\ ferrooxidans} 2Fe^{3+} + H_2O \tag{19.23}$$

The resultant ferric iron then causes chemical oxidation of residual pyrite according to the reaction

$$FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4{}^{2-} + 16H^+ \quad (19.24)$$

whereby Fe^{2+} is regenerated. The bacterial oxidation of Fe^{2+} to Fe^{3+} becomes the rate-controlling reaction in this system (Singer and Stumm, 1970). This is because in the absence of the iron-oxidizing bacteria the rate of Fe^{2+} oxidation is very slow at acid pH. According to a study by Moses et al. (1987), Fe^{3+} is the preferred chemical oxidant of pyrite over O_2, suggesting that an organism like *T. ferrooxidans* will facilitate initiation of pyrite oxidation by generating Fe^{3+} from pyrite [reaction (19.22)].

Schippers and Sand (1999) view the role to *T. ferrooxidans* in the oxidation of pyrite to be essentially indirect by the definition in this book, and they extend this role to the oxidation of other metal sulfides. In their model, *T. ferrooxidans* catalyzes the oxidation of the dissolved Fe^{2+} that results from the chemical oxidation of pyrite by Fe^{3+},

$$FeS_2 + 6Fe^{3+} + 3H_2O \longrightarrow S_2O_3{}^{2-} + 7Fe^{2+} + 6H^+ \quad (19.25)$$

in which thiosulfate is formed as a product. They propose furthermore that the thiosulfate is subsequently oxidized by the ferric iron generated by *T. ferrooxidans*,

$$S_2O_3{}^{2-} + 8Fe^{3+} + 5H_2O \longrightarrow 2SO_4{}^{2-} + 8Fe^{2+} + 10H^+ \quad (19.26)$$

ferric iron being the oxidant in this reaction. At the same time, the thiosulfate is oxidized by *T. ferrooxidans* itself,

$$S_2O_3{}^{2-} + 2O_2 + H_2O \xrightarrow{T.\ ferrooxidans} 2SO_4{}^{2-} + 2H^+ \quad (19.27)$$

In this reaction, oxygen is the terminal electron acceptor (oxidant). It is unclear, however, why thiosulfate should exist at the acid pH (in the range of $pH < 2$ to 3.5) at which these reactions would occur. Thiosulfate is known to decompose into elemental sulfur and sulfite ($SO_3{}^{2-}$) below a pH of 4–5 (see, e.g., Roy and Trudinger, 1970, p. 18).

Sand et al. (1995) believe that direct oxidation of pyrite or any other sulfide mineral by *T. ferrooxidans* does not occur. Their model of bacterial metal sulfide oxidation relies on an abiotic model for pyrite oxidation in which a key reaction is enhanced by the bacteria. This reaction is the oxidation of Fe^{2+} to Fe^{3+}, the Fe^{3+} being needed in the oxidative attack of the pyrite. Their general model suggests that *T. ferrooxidans* cannot oxidize any metal sulfide in the absence of iron in the bulk phase. This is, however, contrary to previous observations (see, e.g., experiments of S. I. Fox summarized by Ehrlich, 1978, p. 71; Nielsen and Beck, 1972; Pogliani et al., 1990). In the case of pyrite, it seems impossible to

distinguish between direct and indirect bacterial mechanisms in the absence of any suppression of the chemical action of ferric iron produced in pyrite oxidation. The ferric iron will always be a product of pyrite oxidation, no matter whether pyrite is attacked directly or indirectly. Sand and collaborators do not distinguish between bulk-phase (dissolved) ferric iron and EPS-bound (complexed) and cell-wall-bound iron of *T. ferrooxidans* in terms of reactivity.

Edwards et al. (2001) approached the question of direct versus indirect action by determining what effect *Thiobacillus ferrooxidans* and *Ferroplasma acidiarmanus* had on pitting of the mineral surface of pyrite, marcasite, and arsenopyrite in a mineral salts medium. They used scanning electron microscopy to make their assessments. They found that extensive pitting on the mineral surface occurred in the absence of bacteria with added ferric chloride but not without it. In the presence of bacteria, its manifestation depended on the kind of bacteria and their attachment, the kind of mineral, and probably other experimental conditions. Thus, *T. ferrooxidans* produced cell-sized and cell-shaped dissolution pits on pyrite but not marcasite or arsenopyrite. *F. acidiarmanus* produced such pits on pyrite and arsenopyrite but not on marcasite. However, individual cells were found in shallow pits on marcasite. The investigators came to the conclusion that overall sulfide dissolution in their experiments was dominated by reaction of a given mineral with bulk-phase Fe^{3+} and not by reaction at the cell/mineral surface interface. Nevertheless, they did not rule out the possibility of a cell/mineral surface interface reaction, but if it did occur they believe it to have been of minor importance in pit formation in these experiments.

If the bound iron (complexed iron) acts as an electron shuttle in the sense of Ingledew (1986), then the bound iron in bacterial cells attached to a pyrite surface may accept the electrons from the half-reactions describing the initial steps in corrosion of pyrite (Rawlings et al., 1999; Moses et al., 1987):

$$FeS_2 + 2H_2O \rightarrow Fe(OH)_2S_2 + 2H^+ + 2e \tag{19.28a}$$

and

$$FeS_2 + 8H_2O \rightarrow Fe^{3+} + 2SO_4{}^{2-} + 16H^+ + 15e \tag{19.28b}$$

In this way, the bacteria take the place of ferric iron in an indirect attack of pyrite. Because *Leptospirillum ferrooxidans* also features bound iron in its EPS, it may also be able to attack pyrite by the direct mechanism when attached to a pyrite surface.

Because iron pyrites usually accompany other metal sulfides in nature, iron pyrite oxidation is an important source of acid for the oxidizing reactions of nonferrous metal sulfides, especially those that consume acid, e.g., the oxidation of chalcocite [reactions (19.15)–(19.17)]. In some cases the host rock in which metal sulfides, including pyrites, are contained may itself consume acid and thus raise the pH of the environment enough to cause extensive precipitation of ferric

iron and thereby prevent oxidation of metal sulfide by it (e.g., Ehrlich, 1977, pp. 149–150).

The foregoing discussion of direct and indirect metal sulfide oxidation dealt mainly with *T. ferrooxidans*, which is capable of the oxidation of both ferrous iron and reduced sulfur. It needs to be extended to *Leptospirillum ferrooxidans*, which oxidizes ferrous iron readily but cannot oxidize reduced sulfur and which is reported to be the dominant member of the leaching microflora in many heap/dump operations (Sand et al., 1992, 1993; Asmah et al., 1999; Bruhn et al., 1999). The ability to oxidize ferrous iron but not reduced sulfur might suggest that *L. ferrooxidans* oxidizes metal sulfides in only an indirect mode. However, like *T. ferrooxidans*, *L. ferrooxidans* produces exopolymer that has an affinity for iron (Gehrke et al., 1995, 1998; Sand et al., 1997). If this bound iron can act like polynuclear iron in *T. ferrooxidans*, *L. ferrooxidans* should also be capable of oxidizing a metal sulfide in a direct mode. A consideration of the mode of action in metal sulfide oxidation also needs to be extended to the other microorganisms that have been found active in metal sulfide oxidation. Most of these can oxidize both Fe^{2+} and reduced forms of sulfur.

19.6 BIOLEACHING OF METAL SULFIDE AND URANINITE ORES

Metal Sulfide Ores

When metal sulfide ore bodies are exposed to moisture and air during mining activities, the ore mineral may begin to undergo gradual oxidation, which may be accelerated by native microorganisms, especially acidophilic iron oxidizers. Groundwater passing through a zone of ore oxidation will pick up soluble products of the oxidation and will issue from the site as acid mine drainage, which contains the metal solubilized by the oxidation. Mining companies harness the microbial oxidizing activity that mobilizes metals in some sulfidic ores on an industrial scale as a means of metal extraction. Such metal bioleaching may be applied to rubblized ore in situ (e.g., McCready, 1988), in ore heaps or dumps, or in special reactors. Initially it was used only with low-grade portions of an ore and with ore tailings, but with improvements in the process it is now also used in treating high-grade ore and ore concentrates.

Low-grade sulfide ores generally contain metal values at concentrations below 0.5% (wt/wt). Their extraction by smelting after milling and subsequent ore enrichment (ore beneficiation) by flotation is uneconomic because of an unfavorable gangue/metal ratio. Years of experience have shown that for most efficient bioleaching, ore heaps should be constructed to heights limited to tens of feet to avoid slumping. They may consist of waste rock or mine tailings, which are the by-products of mining that still contain traces of recoverable metal values

or, in some instances, high-grade ore. The lixiviant can be water, acidified water, or spent acidic leach solution containing ferric sulfate from a previous leaching cycle. It is applied in a fine spray onto the ore heaps or dumps (Fig. 19.3A). The spraying avoids waterlogging of the heaps and dumps, which would exclude needed oxygen (air). Simultaneous diffusion of oxygen into the ore in a heap or dump being leached is important because the microbial leaching process is aerobic. If oxygen does not reach all parts of a heap or dump because its concentration in the leach solution is insufficient to meet the demand for metal sulfide oxidations, anaerobic conditions will develop in those parts that are not reached by oxygen. In such regions, a microbial flora, mostly heterotrophic, has been shown to develop that includes bacteria that reduce ferric to ferrous iron and others that reduce sulfate to H_2S (Fortin et al., 1995; Johnson and Roberto, 1997; Fortin and Beveridge, 1997). The Fe reducers lower the ferric iron concentration available for chemical leaching of metal sulfides in the anoxic zone. The sulfate reducers cause metal species mobilized in the oxidized zones to reprecipitate as sulfides. In some heap leaching operations, pipes are placed in strategic positions within the heap during its construction to optimize access of air to deeper portions of the heap.

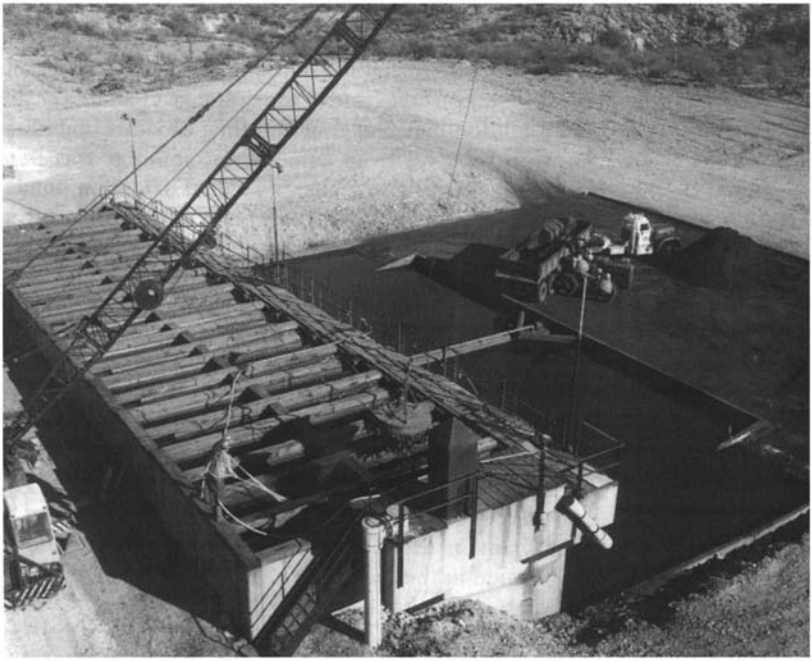
The lixiviant solution applied to the ore makes possible the growth and multiplication of appropriate acidophilic iron oxidizers and the oxidation of pyrite, chalcopyrite, and nonferrous metal sulfides in the ore. Initially solutions issuing from heaps or dumps may be recirculated without any treatment, but ultimately, as microbial and chemical activities continue, the solution in the heaps and dumps becomes charged with dissolved metal values and after issuing from the heaps or dumps is collected as **pregnant solution** in special sumps. It frequently harbors a variety of microorganisms, including autotrophic and heterotrophic bacteria and fungi and protozoa, despite its very acid pH and high metal content (e.g., Ehrlich, 1963b). When the concentration of desired metal values in the pregnant solution in a sump is high enough, they are stripped from the solution. This may be accomplished in one of several ways. A formerly widely used method for copper separation involved treatment of pregnant solution with sponge iron in a specially constructed basin called a launder (Fig. 19.3B). The sponge iron precipitated the copper by **cementation** in a process involving the reaction

$$Cu^{2+} + Fe^0 \rightarrow Cu^0 + Fe^{2+} \tag{19.29}$$

The copper metal formed in this way was very impure and required further refinement by smelting.

After the metal value is stripped from it, pregnant solution is called **barren solution**. It can be recirculated as lixiviant in the leaching operation. However, when cementation was used to strip the copper from pregnant solution, the resultant barren solution became significantly enriched with Fe^{2+}. In many

(A)



(B)

instances it was enriched in ferrous iron to such an extent that some of it had to be removed before the solution could be reintroduced into the leach heaps or dumps. Without removal of the excess ferrous iron, there was a danger of excessive formation of jarosite in the ore heaps or dumps being leached. The jarosite could precipitate on the ore mineral surfaces, interfering with further oxidation, and could also clog the drainage channels in the ore heaps and dumps. Plugging and impeding of the leach process would be the result. Excess iron removal from acid barren solution was best accomplished by bio-oxidation in shallow lagoons called *oxidation ponds*. In these ponds, acidophilic iron oxidizers promoted the oxidation of the ferrous iron with concomitant acidification. A significant portion of the oxidized iron precipitated as basic ferric sulfates, including jarosite. When reintroduced into a heap or dump, the residual iron, mostly ferric, in the treated barren solution caused indirect leaching of the metal sulfides of the ore.

The acid in the recirculated barren solution that entered the heaps and dumps caused relatively rapid weathering of the host rock (gangue) of the ore, resulting in liberation of aluminum from aluminosilicates. This weathering is important in exposing occluded metal sulfide crystals to the lixiviant and the bacteria active in the leaching process. The liberated aluminum may ultimately be separated as $Al(OH)_3$ from pregnant solution by neutralizing it (Zimmerley et al., 1958; Moshuyakova et al., 1971), but this has not been done in practice. The $Al(OH)_3$ could be subsequently used in the manufacture of aluminum metal. High acidity of the lixiviant may play an important role in preventing metal ions formed during leaching from being adsorbed by the host rock (gangue) (Ehrlich, 1977; Ehrlich and Fox, 1967).

A currently preferred method of recovering metal values from pregnant solution involves *electrowinning* if the pregnant solution contains only one major metal value, or *solvent extraction* if several different metal values are present, followed by electrowinning of the separated metal values. Electrowinning

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FIG. 19.3 Bioleaching of copper from sulfide ores. (A) Top of a leach dump showing corrosion-resistant pipes and hose for watering the dump with barren solution. The dark patches represent moistened areas in which oxidation has occurred. Note the thin streams of solution issuing from the hoses in the distance. (B) Launder used in recovering copper by cementation from pregnant solutions from leach dumps. The copper recovered in this way has to be purified by smelting. Currently the preferred method of copper recovery from pregnant solution is electrolysis because it yields a pure product. (Courtesy of Duval Corporation.)

involves deposition of a metal value on a cathode of the metal to be recovered in an electrolytic reaction in which the anode may be made of carbon. The metal product of electrowinning is usually of high purity and normally does not need further refining. These metal separation processes have the advantage of not raising the ferrous iron concentration in barren solution. However, recovery of metal values from pregnant solution by solvent extraction can introduce reagents into the resultant barren solution that are inhibitory to the bacteria involved in leaching.

The acidophilic iron-oxidizing bacteria developing in an ore leaching process play a dual role in solubilizing metal values, as already explained. They generate acid ferric sulfate lixiviant by attacking pyrite and by reoxidizing Fe^{2+}, and they also attack the mineral sulfides directly, as previously discussed. It is usually not possible to assess the extent to which they are involved in direct and indirect oxidation if they are capable of both.

The interior temperature of some ore dumps or heaps, especially if they are not well ventilated, can rise as high as 70–80°C. According to Lyalikova (1960), the heating can be accelerated by bacterial action. The heating is due to the fact that metal sulfide oxidation is an exothermic process. Such a temperature rise is unfavorable for the growth of mesophilic bioleaching bacteria, such as *T. ferrooxidans* and *L. ferrooxidans*, when it occurs in a heap or dump interior. It must be remembered that another important role of these mesophiles in bioleaching of metal sulfides is to regenerate ferric iron in the oxidation ponds, which is not affected by the heat generated in the heaps or dumps.

The observation of interior heating of ore heaps and dumps during leaching suggested to some in the 1960s that the leaching process in dumps and heaps of metal sulfide ores is mostly abiotic. This view changed after the discovery of thermophiles capable of promoting bioleaching of metal sulfides. *T. ferrooxidans* and *L. ferrooxidans*, which are unable to live at temperatures in excess of 37–40°C, are succeeded by acidophilic, iron-oxidizing thermophiles in the interior of leach dumps and heaps where the temperature has risen to the thermophilic range (e.g., Norris, 1997). These microorganisms, which catalyze reactions similar to those of *T. ferrooxidans*, operate optimally at the higher temperatures. Thus, contrary to earlier views, leaching in all parts of a dump or heap is most likely biological. It has also been suggested that the thermophiles may be responsible for regulating the internal temperature of active leach dumps and heaps (Murr and Brierley, 1978).

The primary copper mineral in ore bodies of magmatic hydrothermal origin is chalcopyrite ($CuFeS_2$). This mineral tends to be somewhat refractory to chemical (abiotic) leaching by acidic ferric sulfate compared to the secondary copper sulfide minerals chalcocite (Cu_2S) and covellite (CuS). The oxidation of chalcopyrite in nature can thus be significantly enhanced by *T. ferrooxidans* and

Acidianus brierleyi (Razzell and Trussell, 1963; Brierley, 1974). Ferric iron, when present in excess of 1000 ppm, has been found to inhibit chalcopyrite oxidation (Duncan and Walden, 1972; Ehrlich, 1977), probably because it precipitates as jarosite or adsorbs to the surface of residual chalcopyrite crystals and prevents further oxidation. Bacteria themselves may interfere by generating excess ferric iron from ferrous iron that precipitates or is adsorbed by the residual chalcopyrite.

A typical leach cycle in which copper is recovered either by cementation or by electrowinning is diagrammed in Fig. 19.4. [See Brierley (1978a, 1982), Lundgren and Malouf (1983), and Rawlings (1997a) for further discussion of bacterial leaching.]

In practice, the leaching of PbS through oxidation by *T. ferrooxidans* can present a special problem because the oxidation product, $PbSO_4$, is relatively insoluble. As oxidation of PbS proceeds, $PbSO_4$ is likely to accumulate on the crystal surface and block further access to PbS by bacteria, dissolved Fe^{3+} if present, and oxygen. In the laboratory this problem is largely eliminated when *T. ferrooxidans* oxidizes PbS in batch culture with agitation (Silver and Torma, 1974) or in a large volume of lixiviant in a stirred continuous-flow reactor (Ehrlich, 1988).

Metal sulfide-oxidizing bacteria are naturally associated with metal sulfide-containing deposits, including ore bodies, bituminous coal seams, and the like. They usually exist in a consortium when ore from such deposits is bioleached (Bruhn et al., 1999). Therefore, heap, dump, and in situ leaching operations do not require inoculation with active bacteria, although they may be improved by it (Brierley et al., 1995). Reactor leaching operations, on the other hand, greatly benefit from inoculation with a strain selected for enhanced activity. The inoculum has to be massive for either leaching process in order to outgrow the organisms naturally present on the ore. The ore cannot be sterilized on an industrial scale.

Under natural conditions, growth and activity of the leaching organisms may be limited by one or more restricting environmental factors. These include limited access to an energy source (metal sulfide crystal), limited nitrogen source, unfavorable temperature, and limited access to air and/or moisture (Ehrlich and Fox, 1967; Brock, 1975; Ahonen and Tuovinen, 1989, 1992). It should be noted, however, that *T. ferrooxidans* is capable of anaerobic growth with H_2, formate, or S^0 as electron donor and Fe(III) as terminal electron acceptor (see Chap. 15).

Uraninite Leaching

The principles of bioleaching have also been applied on a practical scale to the leaching of uraninite ores, especially if the ores are low grade. The process may involve dump, heap, or in situ leaching (Wadden and Gallant, 1985; McCready

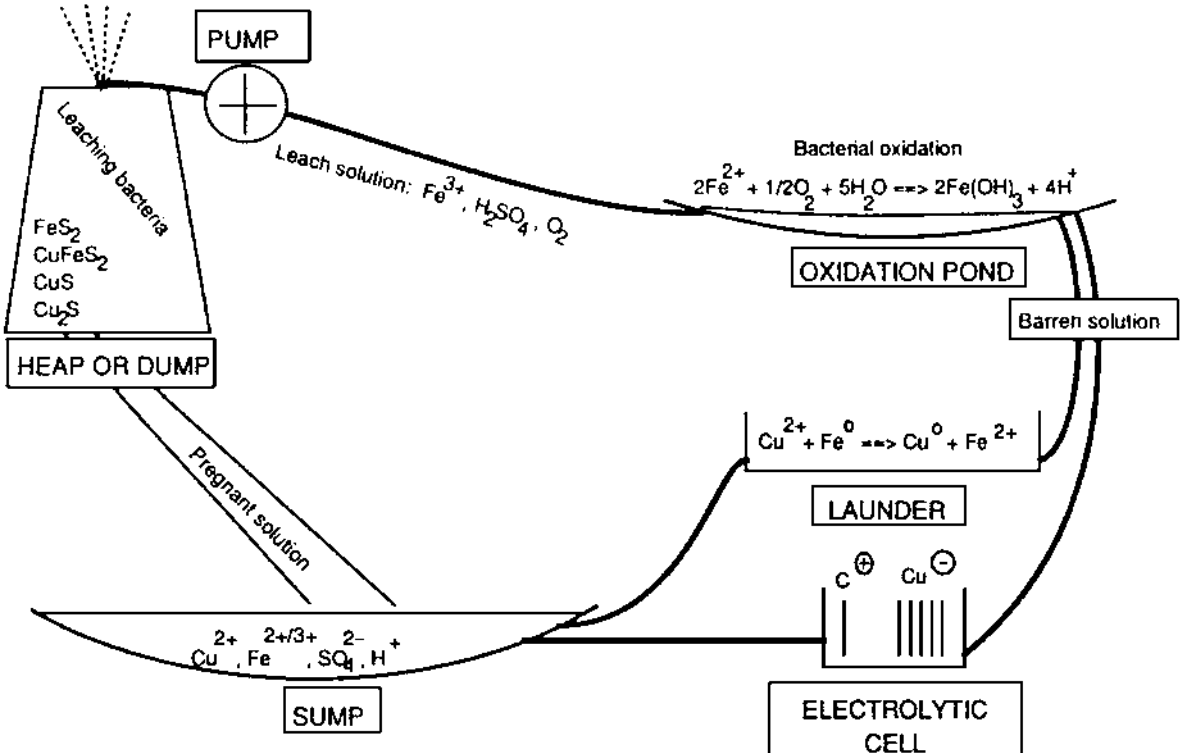


FIG. 19.4 Schematic representation of a bioleach circuit for heap or dump leaching of copper sulfide ore. In addition to copper recovery from pregnant solution by cementation in a launder or by electrowinning, copper can also be recovered by solvent extraction in combination with electrowinning. This is especially useful if the pregnant solution contains two or more base metals.

and Gould, 1990). *Thiobacillus ferrooxidans* is one organism that has been harnessed for this process. Its action in this instance is chiefly indirect by generating an oxidizing lixiviant, acid ferric sulfate, which oxidizes U(IV) in the uraninite to soluble U(VI). The overall reactions leading to uranium mobilization from uraninite can be summarized as follows:

$$2Fe^{2+} + 0.5O_2 + 2H^+ \xrightarrow{T.\ ferrooxidans} 2Fe^{3+} + H_2O \tag{19.30}$$

Some of the resultant ferric iron hydrolyzes:

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+ \tag{19.31}$$

The ultimate product of the hydrolysis will more likely be a basic ferric sulfate such as jarosite rather than ferric hydroxide. The consequence of jarosite formation is that the net yield of acid (protons) will be less than in reaction (19.31) [see reaction (19.21)]. The remaining dissolved ferric iron can then react abiologically with the uraninite to form uranyl ions:

$$UO_2 + 2Fe^{3+} \longrightarrow 2Fe^{2+} + {UO_2}^{2+} \tag{19.32}$$

T. ferrooxidans will reoxidize the ferrous iron from this reaction, thus keeping the process going without the need for continual external resupply of ferric iron to the system. The dissolved uranium may be recovered from solution through concentration by ion exchange.

The acidophilic iron-oxidizing bacteria are often naturally associated with the ore body if it contains pyrite or another form of iron sulfide. Their growth may be stimulated in in situ leaching of depleted mines by intermittent spraying of nutrient-enriched solution onto the floors, walls, and mud of mine stopes (Zajic, 1969). If the leachate becomes anoxic and its pH rises, sulfate-reducing bacteria may develop in it. These bacteria can reprecipitate UO_2 as a result of reaction of ${UO_2}^{2+}$ with H_2S:

$${UO_2}^{2+} + H_2S \longrightarrow UO_2 + 2H^+ + S^0 \tag{19.33}$$

Some observations have given indications that *T. ferrooxidans* can enzymatically catalyze the oxidation of U(IV) to U(VI) and use some of the energy from this reaction to assimilate CO_2 (DiSpirito and Tuovinen, 1981, 1982a, 1982b) (see also Chap. 17). Living cells accumulate significantly less uranium than dead cells and bind it chiefly in their cell envelope (DiSpirito et al., 1983). Despite this capacity of *T. ferrooxidans* to oxidize U(IV) directly, bioleaching of uraninite is believed to involve chiefly the indirect mechanism.

Uranium bioleaching is another example of a natural process that is artificially stimulated. Under natural conditions, the reactions described here must occur on a very limited scale and thus cause only slow mobilization of uranium.

Uranium in drainage from uranium mines can be microbiologically precipitated by its reduction under anaerobic conditions from dissolved UO_2^{2+} to insoluble UO_2. Some sulfate-reducing bacteria are capable of this action (Lovley et al., 1993). *Geobacter metallireducens* and *Shewanella putrefaciens* are two other bacterial species capable of precipitating U(IV) under anaerobic conditions (Gorby and Lovley, 1992). The electron donors used by the bacteria in these reactions are usually organic compounds but can be H_2 in the case of some sulfate reducers and *Shewanella*. This activity can be useful in remediating uranium-containing mine drainage, although only at circumneutral pH.

Mobilization of Uranium in Granitic Rocks by Heterotrophs

Thiobacillus ferrooxidans is not the only organism capable of uranium mobilization. Heterotrophic microorganisms such as some members of the soil microflora and bacteria from granites or mine waters (*Pseudomonas fluorescens, P. putida, Achromobacter*) can mobilize uranium in granitic rocks, ore, and sand by weathering through mineral interaction with organic acids and chelators produced by the microorganisms (Magne et al., 1973, 1974; Zajic, 1969). Magne et al. found experimentally that the addition of thymol to percolation columns of uraniferous material fed with glucose solution selected a microbial flora whose efficiency in uranium mobilization was improved by greater production of oxalic acid. The authors suggested that in nature, phenolic and quinoid compounds of plant origin can serve the role of thymol. They also reported that microbes can precipitate uranium by digestion of soluble uranium complexes (Magne et al., 1974), i.e., by microbial destruction of the organic moiety that complexes the uranium. These observations may explain how in nature uranium in granitic rock may be mobilized by bacteria and reprecipitated and concentrated elsewhere under the influence of other microbial activity.

Study of Bioleaching Kinetics

A number of studies have been published on the kinetics of bioleaching metal sulfides under controlled conditions in the presence of ferrous iron. They include studies by Boon et al. (1995), Hansford (1997), Hansford and Vargas (1999), Crundwell (1995, 1997), Nordstrom and Southam (1997), Driessens et al. (1999), Fowler and Crundwell (1999), and Howard and Crundwell (1999). In this work the assumption is made or the inference is drawn that bioleaching of metal sulfides proceeds in only one mode. The existence of separate direct and indirect modes that may occur concurrently in the same leaching operation appears to be

rejected. However, Fowler and Crundwell (1999) assign oxidation of S^0 that appears at the surface of ZnS during leaching to attached *T. ferrooxidans* cells.

Industrial Versus Natural Bioleaching

Industrial bacterial leaching of metal sulfide ores harnesses naturally occurring microbiological processes by creating selective and optimized conditions that allow leaching to occur at fast rates. In the absence of human intervention, the same processes occur only at very slow rates in highly localized situations, contributing in the case of sulfide ore to a very slow, gradual change from reduced to oxidized ore. This accounts for the relative stability of undisturbed ore bodies.

19.7 BIOEXTRACTION OF METAL SULFIDE ORES BY COMPLEXATION

Some metal sulfide ores cannot be oxidized by acidophilic iron-oxidizing bacteria because they contain too great an amount of acid-consuming constituents in the host rock (gangue). The metals in such ores may be amenable to extraction by some microorganisms such as fungi (e.g., Burgstaller and Schinner, 1993). Wenberg et al. (1971) reported the isolation of the fungus *Penicillium* sp. from a mine tailings pond of the White Pine Copper Co. in Michigan that produced unidentified metabolites in Czapek's broth containing sucrose, $NaNO_3$, and cysteine, methionine, or glutamic acid that could mobilize copper from sedimentary ores of the White Pine deposit. *T. ferrooxidans* could not be employed for leaching of this ore because of the presence of significant quantities of calcium carbonate that would neutralize the required acid. Similar findings were reported by Hartmannova and Kuhr (1974), who found that not only *Penicillium* sp. but also *Aspergillus* sp. (e.g., *A. niger*) were active in producing complexing compounds that leached copper. More recently, Mulligan and Galvez-Cloutier (2000) demonstrated the mobilization of copper in an oxidized mining residue by *A. niger* in a sucrose–mineral salts medium. The chief mobilizing agents produced by the fungus were gluconic and citric acids, which can act as acidulants as well as ligands of metal ions.

Wenberg et al. (1971) grew their fungus in the presence of copper ore (sulfide or native copper minerals with basic gangue constituents). The addition of some citrate to the medium lowered the toxicity of the extracted copper when the fungus was grown in the presence of the ore. They obtained better results when the fungus was grown in the absence of the ore and the ore was then treated with the spent medium from the fungus culture. The principle of action of the fungi in all the cited experiments is similar to that involved in a study by Kee and Bloomfield (1961), who noted the dissolution of the oxides of several trace

elements (e.g., ZnO, PbO_2, MnO_2, CoO, Co_2O_3) with anaerobically fermented plant material (Lucerne and Cocksfoot). The principle of action is also similar to that employed in the experiments of Parès (1964a, 1964b, 1964c), in which *Serratia marcescens, Bacillus subtilis, B. sphaericus* and *B. firmus* solubilized copper and some other metals that were associated with laterites and clays. The bacteria generated appropriate ligands in special culture media. The ligands extracted the metals from the ores by forming chelates, which are more stable than the original insoluble form of the metals in the ores. This type of reaction can be formulated as follows:

$$MA + HCh \longrightarrow MCh + H^+ + A^- \tag{19.34}$$

where MA is a metal salt (mineral), HCh a ligand (chelating agent), MCh the resultant metal chelate, and A^- the counter ion of the original metal salt, S^{2-} in the case of metal sulfides. The S^{2-} may undergo chemical or bacterial oxidation. The use of carboxylic acids in industrial leaching of ores has been proposed as a general process (*Chemical Processing*, 1965).

19.8 FORMATION OF ACID COAL MINE DRAINAGE

When bituminous coal seams that contain pyrite inclusions are exposed to air and moisture during mining, the pyrites undergo oxidation, leading to the formation of acid mine drainage (AMD). With the onset of pyrite oxidation, iron-oxidizing thiobacilli become readily detectable in the drainage (Leathen et al, 1953). *Thiobacillus thiooxidans* also makes an appearance. When *T. ferrooxidans* is involved, pyrite bio-oxidation proceeds by the reactions previously described [reactions (19.19)–(19.24)]. *T. thiooxidans*, which cannot oxidize ferrous iron, probably oxidizes elemental sulfur and other partially reduced forms of sulfur, which may form as intermediates in pyrite oxidation, to sulfuric acid (e.g., Mustin et al., 1992, 1993) [reaction (19.17)]. The chief products of pyrite oxidation are thus sulfuric acid and basic ferric sulfate (jarosite and amorphous basic ferric sulfates). Streams that receive this mine drainage may exhibit pH values ranging from 2 to 4.5 and sulfate ion concentrations ranging from 1000 to 20,000 mg L^{-1} but a nondetectable ferrous iron concentration (Lundgren et al., 1972). Walsh and Mitchell (1972b) proposed that a *Metallogenium*-like organism that has been isolated from AMD may be the dominant iron-oxidizing organism attacking the pyrite in exposed pyrite-containing coal seams until the pH drops below 3.5. After that, the more acid-tolerant *T. ferrooxidans* and probably *Leptospirillum ferrooxidans* take over. The *Metallogenium*-like organism does not, however, appear to be essential to lower the pH in a pyritic environment to make it favorable for the acidophilic iron oxidizers. *T. ferrooxidans* itself may be capable

of doing that, at least in pyrite-containing coal or overburden (Kleinmann and Crerar, 1979). It may accomplish this by initial direct attack of pyrite [see reaction (19.22)], creating an acid microenvironment from which the organism and the acid it generates spread.

An early study of microbial succession in coal spoil under laboratory conditions was carried out by Harrison (1978). He constructed an artificial coal spoil by heaping a homogeneous mixture of 1 part crushed, sifted coal plus 2 parts shale and 8 parts subsoil from the overburden of a coal deposit into a mound 50 cm in diameter and 25 cm high on a plastic tray. The mound was inoculated with 20 L of an emulsion of acid soil, drainage water, and mud from a spoil from an old coal strip mine, which was poured on the bottom of the plastic tray. The inoculum was absorbed by the mound and migrated upward, presumably by capillary action. Evaporation losses during the experiment were made up by periodic additions of distilled water to the free liquid on the tray.

Initial samples taken at the base of the mound yielded evidence of the presence of heterotrophic bacteria. These bacteria were dominant and reached a population density of about 10^7 cells g^{-1} within 2 weeks. After 8 weeks, heterotrophs were still dominant, although the pH had dropped from 7 to 5. Between 12 and 20 weeks, the population decreased by about an order of magnitude, coinciding with a slight decrease in pH to just below pH 5 caused by a burst of growth by sulfur-oxidizing bacteria, which then died off progressively. Thereafter the heterotrophic population increased again to just below $10^7\,g^{-1}$. In samples from near the summit of the mound, heterotrophs also predominated for the first 15 weeks but then decreased dramatically from 10^6 to 10^2 cells g^{-1}, concomitant with a drop in pH to 2.6. The pH drop was correlated with a marked rise in the population density of sulfur- and iron-oxidizing autotrophic bacteria (*T. thiooxidans and T. ferrooxidans*), the former dominating briefly over the latter in the initial weeks. Protozoans, algae, an arthropod, and a moss were also noted, mostly at the higher pH values. *Metallogenium* of the type of Walsh and Mitchell (1972a) was not seen.

The sulfur-oxidizing bacteria were assumed to be making use of elemental sulfur resulting from the oxidation of pyrite by ferric sulfate:

$$FeS_2 + Fe_2(SO_4)_3 \rightarrow 3FeSO_4 + 2S^0 \tag{19.35}$$

More specifically, as Mustin et al. (1992, 1993) indicated, the sulfur may arise as a result of anodic reactions at the surface of pyrite crystals. It is also possible that at least some of the sulfur arises indirectly from the reduction of microbial sulfate in anaerobic zones of the coal spoil. The reduction of sulfate would yield H_2S, which then becomes the energy source for thiobacilli such as *T. thioparus* that oxidize it to sulfur at the interface of the oxidized and reduced zones, provided the ambient pH is not too far below neutrality. Anaerobic bacteria were not sought in this study.

After 7 weeks of incubation, a mineral efflorescence developed on the surface of the mound. It consisted mainly of sulfates of Mg, Ca, Na, Al, and Fe. The magnesium sulfate was in the form of a hexahydrate rather than epsomite. The metals were leached from the coal, but magnesium was also leached from the overburden material. Harrison's study, which was reported in 1978 before the introduction of the techniques of molecular biology to microbial ecology, deserves to be repeated using molecular techniques. It could yield new insights into the microbial successions in the development of AMD from coal spoils.

Darland et al. (1970) isolated a thermophilic, acidophilic *Thermoplasma acidophilum* from a coal refuse pile that had become self-heated. The organism lacks a true bacterial cell wall and resembles mycoplasmas. It is an archaeon. Its growth temperature optimum was 59°C (range 45–62°C), and its optimum pH for growth was between 1 and 2 (pH range 0.96–3.5). The organism is a heterotroph, growing readily in a medium of 0.02% $(NH_4)_2SO_4$, 0.05% $MgSO_4$, 0.025% $CaCl_2 \cdot 2H_2O$, 0.3% KH_2PO_4, 0.1% yeast extract, and 1.0% glucose at pH 3.0. Its relation to the coal environment and its contribution, if any, to the acid mine drainage problem needs to be clarified.

New Discoveries Relating to Acid Mine Drainage

A recent study of abandoned mines at Iron Mountain, California, resulted in a startling discovery insofar as the generation of acid mine drainage is concerned, at least at this mine. The ore body at Iron Mountain at the time it was mined contained various metal sulfides and was a source of Fe, Cu, Ag, and Au. A significant part of the iron was in the form of pyrite. The drainage currently coming from abandoned mine workings contains varying amounts of these metals as well as Cd and is very acid. A survey of the distribution of *T. ferrooxidans* and *L. ferrooxidans* in sediments and solutions from a pyrite deposit in the Richmond Mine, seepage from a tailings pile, and AMD storage tanks outside this mine was undertaken (Schrenk et al., 1998). It revealed that *T. ferrooxidans* occurred in slime-based communities at $pH > 1.3$ at temperatures below 30°C whereas *L. ferrooxidans* was abundant in subsurface slime-based communities and also occurred in planktonic form at pH values in the range of 0.3–0.7 between 30 and 50°C (Fig. 19.5). *T. ferrooxidans* appeared to affect precipitation of ferric iron but seemed to have a minor role in acid generation. Neither *T. ferrooxidans* nor *L. ferrooxidans* was thought to exert a direct catalytic effect on metal sulfide oxidation, but they were each thought to play an active role in generating ferric iron as an oxidizing agent (Schrenk et al., 1998). Microbiological investigation of underground areas (drifts) in the Richmond Mine revealed the presence of Archaea as well as *T. ferrooxidans* and *L. ferrooxidans* (Edwards et al., 1999a), but only the Archaea and *L. ferrooxidans* were associated with acid-generating sites (Edwards et al., 1999b). The proportions in which they were detected varied with the site and the season of the year. Members of the Bacteria

FIG. 19.5 A field site in an abandoned stope within the Richmond Mine at Iron Mountain in northern California, showing streamers of bacterial slime in the foreground. A pH electrode is visible on the right. A fishing line in the center secures a white bottle with a filter lid that contains sulfide mineral samples to study their fate in situ. (Courtesy of Thomas Gihring.)

were most abundant during winter months when Archaea were nearly undetectable. The reverse was found in summer and fall months, when Archaea represented ~50% of the total population. The authors correlated these population fluctuations with rainfall and conductivity (dissolved solids), pH and temperature of the mine water. As noted above, *T. ferrooxidans* was the least pH-tolerant and was less temperature-tolerant than *L. ferrooxidans*. As already mentioned, *T. ferrooxidans* was absent at acid-generating sites in the mine (Edwards et al., 1999b). Any attachment of iron-oxidizing bacteria was restricted to pyrite phases. Based on dissolution rate measurements, the investigators found that attached and planktonic species contributed comparably to acid release. Among the Archaea, a newly discovered microbe, *Ferroplasma acidarmanus*, grew in slime streamers on the surface of pyrite surfaces (Edwards et al., 2000). It constituted up to 85% of the total communities in the slimes and sediments that were examined. In laboratory study, it was found to be extremely acid-tolerant. It was able to grow at pH 0 and exhibited a pH optimum at 1.2 (see also earlier section). Its cells lack a wall. It belongs to the Archaean order Thermoplasmales (Edwards et al., 2000) and is a close relative of *Ferroplasma acidophilum* (Golyshina et al., 2000). On the basis of studies to date, Edwards et al. (2001) believe that *F. acidarmanus* and *F. acidophilum* promote pyrite oxidation by generating the oxidant Fe^{3+} from Fe^{2+} (indirect mechanism).

Using molecular phylogenetic techniques, examination of a ~1 cm thick slime on finely disseminated pyrite ore collected in the Richmond Mine revealed the presence of a variety of bacterial types (Bond et al., 2000). Predominant were *Leptospirillum* species representing 71% of the clones recovered. *Acidimicrobium*-related species, including "*Ferromicrobium acidophilus*", were detected. Archaea were represented by Thermoplasmales. They included organisms closely related to *Ferroplasma acidophilum* and *F. acidarmanus* and organisms with an affinity to *Thermoplasma acidophilum*. Also detected were members of the delta subdivision of sulfate and metal reducers. These findings indicate the presence of microniches in the slime, some supporting aerobic (oxidizing) activity, others anaerobic (reducing) activity.

The interesting studies at Iron Mountain relegate the position of *T. ferrooxidans* in acid mine drainage formation to the periphery of the remaining ore body at the present time. This position appears to be dictated by prevailing environmental conditions inside and outside the mine. This may not always have been the position of *T. ferrooxidans*. Its current position may be the result of a species succession in microbial community development that started when the pyrite and other metal sulfides in the ore body first began to be oxidized upon exposure to air and water as mining proceeded. The findings from Iron Mountain raise the question of whether similar changes have occurred or are taking place in the generation of AMD from bituminous coal mines where the pyrite in the coal is more dispersed than in the Iron Mountain ore body. Clearly, AMD formation

from bituminous coal mines needs to be reinvestigated. The question of microbial succession in the development of AMD in the mining of metal sulfide ore bodies also needs to be investigated.

19.9 SUMMARY

Metal sulfides may occur locally in high concentrations, in which case they constitute ores. Although most nonferrous sulfides are formed abiogenically through magmatic and hydrothermal processes, a few sedimentary deposits are of biogenic origin. More important, some sedimentary ferrous sulfide accumulations are biogenically formed. The microbial role in biogenesis of any of these sulfide deposits is the genesis of H_2S, usually from the bacterial reduction of sulfate, but in a few special cases possibly from the mineralization of organic sulfur compounds. Because metal sulfides are relatively insoluble, spontaneous reaction of metal ions with the biogenic sulfide proceeds readily. Biogenesis of specific metal sulfide minerals has been demonstrated in the laboratory. These experiments require relatively insoluble metal compounds as starting materials to limit the toxicity of the metal ions to the sulfate-reducing bacteria. In nature, adsorption of the metal ions by sediment components serves a similar function in lowering their concentration below their toxic levels for sulfate reducers.

Metal sulfides are also subject to oxidation by bacteria such as *Thiobacillus ferrooxidans, Leptospirillum ferrooxidans, Sulfolobus* spp., *Acidianus brierleyi*, and others. The bacterial action may involve direct oxidative attack of the crystal lattice of a metal sulfide or indirect oxidative attack by generation of a lixiviant (acid ferric sulfate), which oxidizes the metal sulfide chemically. The indirect mechanism is of primary importance in the solubilization of uraninite (UO_2). Microbial oxidation of metal sulfides is industrially exploited in extracting metal values from low-grade metal sulfide ore and uraninite and is being tested successfully on some high-grade ore and ore concentrates. In bituminous coal seams that are exposed as a result of mining activity, pyrite oxidation by these bacteria is an industrially deleterious process; it is the source of harmful acid mine drainage.

REFERENCES

Ahonen L, Tuovinen OH. 1989. Microbiological oxidation of ferrous iron at low temperatures. Appl Environ Microbiol 55:312–316.

Ahonen L, Tuovinen OH. 1992. Bacterial oxidation of sulfide minerals in column leaching experiments at suboptimal temperatures. Appl Environ Microbiol 58:600–606.

Asmah RH, Bosompem KM, Osei YD, Rodriguez FK, Addy ME, Clement C, Wilson MD. 1999. Isolation and characterization of mineral oxidizing bacteria from the Obuasi gold mining site, Ghana. In: Amils R, Ballester A, eds. Biohydrometallurgy and the

Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 657–662.
Baas Becking LGM, Moore D. 1961. Biogenic sulfides. Econ Geol 56:259–272.
Bagdigian RM, Myerson AS. 1986. The adsorption of *Thiobacillus ferrooxidans* on coal surfaces. Biotech Bioeng 28:467–479.
Balashova, VV, Vedenina IYa, Markosyan GE, Zavarzin GA. 1974. The autotrophic growth of *Leptospirillum ferrooxidans*. Mikrobiologiya 43:581–585 (Engl transl, pp 491–494).
Ballard RD, Grassle JF. 1979. Strange world without sun. Return to oases of the deep. Natl Geogr Mag 156:680–703.
Bennett JC, Tributsch H. 1978. Bacterial leaching patterns of pyrite crystal surfaces. J Bacteriol 134:310–317.
Berner RA. 1984. Sedimentary pyrite formation: An update. Geochim Cosmochim Acta 48:605–615.
Bhatti TM, Gigham JM, Carlson L, Tuovinen OH. 1993. Mineral products of pyrrhotite oxidation by *Thiobacillus ferrooxidans*. Appl Environ Microbiol 59:1984–1990.
Bonatti E. 1972. Authigenesis of minerals—marine. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 48–56.
Bonatti E. 1978. The origin of metal deposits in the oceanic lithosphere. Sci Am 238:54–61.
Bond PL, Smriga SP, Banfield JF. 2000. Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. Appl Environ Microbiol 66:3842–3849.
Boon M, Hansford GS, Heijnen JJ. 1995. The role of bacterial ferrous iron oxidation in the bio-oxidation of pyrite. In: Vargas T, Jerez CA, Wiertz JV, Toledo H, eds. Biohydrometallurgical Processing, Vol I. Santiago, Chile: Univ Chile, pp. 153–163.
Brierley CL. 1974. Leaching. Use of a high-temperature microbe. In: Solution Mining Symposium. Proc 103rd AIME Annu Meet, Dallas, TX, Feb 25–27, 1974, pp 461–469.
Brierley CL 1978a. Bacterial leaching. CRC Crit Rev 6:107–262.
Brierley JA. 1978b. Thermophilic iron-oxidizing bacteria found in copper leaching dumps. Appl Environ Microbiol 36:523–525.
Brierley CL. 1982. Microbiological mining. Sci Am 247:44–53, 150.
Brierley CL, Brierley JA. 1973. A chemoautotrophic and thermophilic microorganism isolated from an acid hot spring. Can J Microbiol 19:183–188.
Brierley CL, Murr LE. 1973. Leaching: Use of a thermophilic chemoautotrophic microbe. Science 179:488–490.
Brierley JA, Lockwood SJ. 1977. The occurrence of thermophilic iron-oxidizing bacteria in a copper leaching system. FEMS Microbiol Lett 2:163–165.
Brierley JA, Norris PR, Kelly DP, LeRoux NW. 1978. Characteristics of a moderately thermophilic and acidophilic iron-oxidizing *Thiobacillus*. Eur J Appl Microbiol Biotechnol 5:291–299.
Brierley JA, Wan RY, Hill DL, Logan TC. 1995. Biooxidation-heap pretreatment technology for processing lower grade refractory gold ores. In: Vargas T, Jerez CA, Wiertz JV, Toledo H, eds. Biohydrometallurgical Processing, Vol I. Santiago, Chile: Univ Chile, pp 253–262.

Brock TD. 1975. Effect of water potential on growth and iron oxidation by *Thiobacillus ferrooxidans*. Appl Microbiol 29:495–501.

Bruhn DF, Thompson DN, Noah KS. 1999. Microbial ecology assessment of a mixed copper oxide/sulfide dump leach operation. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 799–808.

Bryner LC, Anderson R. 1957. Microorganisms in leaching sulfide minerals. Ind Eng Chem 49:1721–1724.

Bryner LC, Jameson AK. 1958. Microorganisms in leaching sulfide minerals. Appl Microbiol 6:281–287.

Bryner LC, Beck JV, Davis BB, Wilson DG. 1954. Microorganisms in leaching sulfide minerals. Ind Eng Chem 46:2587–2592.

Burgstaller W, Schinner F. 1993. Leaching of metals with fungi. J Biotechnol 27:91–116.

Cameron EM. 1982. Sulfate and sulfate reduction in early Precambrian oceans. Nature (Lond) 296:145–148.

Carlson L, Lindström EB, Hallberg KB, Tuovinen OH. 1992. Solid-phase products of bacterial oxidation of arsenical pyrite. Appl Environ Microbiol 58:1046–1049.

Chemical Processing. 1965. Carboxylic acids in metal extraction. Chem Proc 11:24–25.

Chen C-Y, Skidmore DR. 1987. Langmuir adsorption isotherm for *Sulfolobus acidocaldarius* on coal particles. Biotech Lett 9:191–194.

Chen C-Y, Skidmore DR. 1988. Attachment of *Sulfolobus acidocaldarius* cells in coal particles. Biotechnol Progr 4:25–30.

Corliss JB, Dymond J, Gordon LI, Edmond JM, von Herzen RP, Ballard RD, Green K, Williams D, Bainbridge A, Crane K, van Andel TH. 1979. Submarine thermal springs on the Galapagos Rift. Science 203:1073–1083.

Crundwell FK. 1995. Mathematical modeling and optimisation of bacterial leaching plants. In: Vargas T, Jerez CA, Wiertz JV, Toledo H, eds. Biohydrometallurgical Processing; Vol 1. Santiago, Chile: Univ Chile, pp 437–446.

Crundwell FK. 1997. Physical chemistry of bacterial leaching. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 13–26.

Cuthbert ME. 1962. Formation of bornite at atmospheric temperature and pressure. Econ Geol 57:38–41.

Darland G, Brock TD, Samsonoff W, Conti SF. 1970. A thermophilic, acidophilic mycoplasma isolated from a coal refuse pile. Science 170:1416–1418.

Davidson CF. 1962a. The origin of some strata-bound sulfide ore deposits. Econ Geol 57:265–274.

Davidson CF. 1962b. Further remarks on biogenic sulfides. Econ Geol 57:1134–1137.

Dévigne J-P. 1968a. Précipitation du sulfure de plomb par un micrococcus tellurique. CR Acad Sci (Paris) 267:935–937.

Dévigne J-P. 1968b. Une bactérie saturnophile, Sarcina flava Bary 1987. Arch Inst Pasteur (Tunis) 45:341–358.

Dévigne J-P. 1973. Une métallogenèse microbienne probable en milieux sédimentaires: Celle de la galéne. Cah Geol 89:35–37.

Dew DW, van Buuren C, McEwan K, Bowker C. 1999. Bioleaching of base metal sulfide concentrates: A comparison of mesophile and thermophile bacterial cultures. In: Amils

R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 229–238.
DiSpirito AA, Tuovinen OH. 1981. Oxygen uptake coupled with uranous sulfate oxidation by *Thiobacillus ferrooxidans* and *T. acidophilus*. Geomicrobiol J 2:275–291.
DiSpirito AA, Tuovinen OH. 1982a. Uranous ion oxidation and carbon dioxide fixation by *Thiobacillus ferrooxidans*. Arch Microbiol 133:28–32.
DiSpirito AA, Tuovinen OH. 1982b. Kinetics of uranous and ferrous iron oxidation by *Thiobacillus ferrooxidans*. Arch Microbiol 133:33–37.
DiSpirito AA, Talnagi JW Jr, Tuovinen OH. 1983. Accumulation and cellular distribution of uranium in *Thiobacillus ferrooxidans*. Arch Microbiol 135:250–253.
Donati E, Curutchet G, Pogliani C, Tedesco P. 1995. Bioleaching of covellite by individual or combined cultures of *Thiobacilllus ferrooxidans* and *Thiobacillus thiooxidans*. In: Vargas T, Jerez CA, Wiertz JV, Toledo H, eds. Biohydrometallurgical Processing, Vol 1. Santiago, Chile: Univ Chile, pp 91–99.
Driessens YPM, Fowler TA, Crundwell FK. 1999. A comparison of the bacterial and chemical leaching of sphalerite at the same solution conditions. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 201–208.
Duncan DW, Walden CC. 1972. Microbial leaching in the presence of ferric iron. Dev Ind Microbiol 13:66–75.
Duncan DW, Landesman J, Walden CC. 1967. Role of *Thiobacillus ferrooxidans* in the oxidation of sulfide minerals. Can J Microbiol 13:397–403.
Edmond JM, Von Damm KL, McDuff RE, Measures CI. 1982. Chemistry of hot springs on the East Pacific Rise and their effluent dispersal. Nature (Lond) 297:187–191.
Edwards KJ, Schrenk MO, Hamers R, Banfield JF. 1998. Mineral oxidation of pyrite: Experiments using microorganisms from an extreme acidic environment. Am Mineral 83:1444–1453.
Edwards KJ, Gihring TM, Banfield JF. 1999a. Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. Appl Environ Microbiol 65:3627–3632.
Edwards KJ, Goebell BM, Rodgers RM, Schrenk MO, Gihring TM, Cardona MM, Hu B, McGuire MM, Hamers RJ, Pace NR. 1999b. Geomicrobiology of pyrite (FeS_2) dissolution: Case study at Iron Mountain, California. Geomicrobiol J 16:155–179.
Edwards KJ, Bond PL, Gihrig TM, Banfield JF. 2000. An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. Science 287:1796–1799.
Edwards KJ, Hu B, Hamers RJ, Banfield JF. 2001. A new look at patterns on sulfide minerals. FEMS Microbiol Ecol 34:197–206.
Ehrlich HL. 1963a. Bacterial action on orpiment. Econ Geol 58:991–994.
Ehrlich HL. 1963b. Microorganisms in acid drainage from a copper mine. J Bacteriol 86:350–352.
Ehrlich HL. 1964. Bacterial oxidation of arsenopyrite and enargite. Econ Geol 59:1306–1312.
Ehrlich HL. 1977. Bacterial leaching of low-grade copper sulfide ore with different lixiviants. In: Schwartz W, ed. Conference Bacterial Leaching. Gesellschaft für Biotechnologische Forschung mbH, Braunschweig-Stöckheim. Weinstein, Germany: Verlag Chemie, pp 145–155.

Ehrlich HL. 1978. Inorganic energy sources for chemolithotrophic and mixotrophic bacteria. Geomicrobiol J 1:65–83.

Ehrlich HL. 1988. Bioleaching of silver from a mixed sulfide ore in a stirred reactor. In: Norris PR, Kelly DP, eds. Biohydrometallurgy. Kew, Surrey, UK: Sci Technol Letters, pp 223–231.

Ehrlich HL. 1993a. Electron transfer from acetate to the surface of MnO_2 particles by a marine bacterium. J Ind Microbiol 12:121–128.

Ehrlich HL. 1993b. A possible mechanism for the transfer of reducing power to insoluble mineral oxide in bacterial respiration. In: Torma AE, Apel ML, Brierley CL, eds. Biohydrometallurgical Technologies. Warrendale, PA: Minerals, Metals Mater Soc, pp 415–422.

Ehrlich HL. 2000. Past, present, and future in biohydrometallurgy. Hydrometallurgy 59:127–134.

Ehrlich HL, Fox SI. 1967. Environmental effects on bacterial copper extraction from low-grade copper sulfide ores. Biotech Bioeng 9:471–485.

Ehrlich HL, Ingledew WJ, Salerno JC. 1991. Iron- and manganese-oxidizing bacteria. In: Shively JM, Barton LL, eds. Variations in Autotrophic Life. London: Academic Press, pp 147–170.

Fenchel T, Blackburn TH. 1979. Bacteria and Mineral Cycling. London: Academic Press.

Fortin D, Beveridge TR. 1997. Microbial sulfate reduction within sulfidic mine tailings: Formation of diagenetic Fe sulfides. Geomicrobiol J 14:1–21.

Fortin D, Davis B, Southam G, Beveridge TJ. 1995. Biogeochemical phenomena induced by bacteria within sulfidic mine tailings. J Ind Microbiol 14:178–185.

Fowler TA, Crundwell FK. 1999. Leaching zinc sulfide by *Thiobacilllus ferrooxidans*: Bacterial oxidation of the sulfur product layer increases the rate of zinc sulfide dissolution at high concentrations of ferrous ions. Appl Environ Microbiol 65:5285–5292.

Fox SI. 1967. Bacterial oxidation of simple copper sulfides. PhD Thesis. Rensselaer Polytechnic Inst, Troy, NY.

Freke AM, Tate D. 1961. The formation of magnetic iron sulfide by bacterial reduction of iron solutions. J Biochem Microbiol Technol Eng 3:29–39.

Garcia O Jr, Bigham JM, Tuovinen OH. 1995. Oxidation of galena by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Can J Microbiol 41:508–514.

Gehrke T, Hallmann R, Sand W. 1995. Importance of exopolymers from *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* for bioleaching. In: Vargas T, Jerez CA, Wiertz KV, Toledo H, eds. Biohydrometallurgical Processing, Vol. 1. Santiago, Chile: Univ Chile, pp. 1–11.

Gehrke T, Telegdi J, Thierry D, Sand W. 1998. Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. Appl Environ Microbiol 64:2743–2747.

Giblin AE. 1988. Pyrite formation in marshes during early diagenesis. Geomicrobiol J 6:77–97.

Giblin AE, Howarth RW. 1984. Porewater evidence for a dynamic sedimentary iron cycle in salt marshes. Limnol Oceanogr 29:47–63.

Golovacheva RS, Karavaiko GI. 1979. A new genus of thermophilic spore-forming bacteria, *Sulfobacillus*. Mikrobiologiya 47:815–822 (Engl. transl, pp 658–665).

Golyshina OV, Pivovarova TA, Karavaiko GI, Kondrat'eva TF, Moore RB, Abraham W-R, Lunsdorf H, Timmis KN, Yakimov MM, Golyshin PN. 2000. *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the Ferroplasmaceae fam. nov., comprising a distinct lineage of the Archaea. Int J Syst Evol Microbiol 50:997–1006.

Gorby YA, Lovley DR. 1992. Enzymatic uranium precipitation. Environ Sci Technol 26:205–207.

Hallberg RO. 1978. Metal-organic interactions at the redoxcline. In: Krumbein WE, ed. Environmental Biogeochemistry and Geomicrobiology, Vol 3. Methods, Metals, and Assessment. Ann Arbor, MI: Ann Arbor Sci, pp 947–953.

Hansford GS. 1997. Recent developments in modeling the kinetics of bioleaching. In: Rawlings DE, ed. Biomining: Theory, Microbes and Industrial Processes. Berlin, Germany: Springer-Verlag, pp 153–175.

Hansford GS, Vargas T. 1999. Chemical and electrochemical basis of bioleaching processes. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 13–26.

Harrison AP Jr. 1978. Microbial succession and mineral leaching in an artificial coal spoil. Appl Microbiol 36:861–869.

Harrison AP Jr., Norris PR. 1985. *Leptospirillum ferrooxidans* and similar bacteria: Some characteristics and genomic diversity. FEMS Microbiol Lett 30:99–102.

Hartmannova V, Kuhr I. 1974. Copper leaching by lower fungi. Rudy 22:234–238.

Howard D, Crundwell FK. 1999. A kinetic study of the leaching of chalcopyrite with Sulfolobus metallicus. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 209–217.

Howarth RW. 1979. Pyrite: Its rapid formation in a salt marsh and its importance in ecosystem metabolism. Science 203:49–51.

Howarth RW, Merkel S. 1984. Pyrite formation and the measurement of sulfate reduction in salt marsh sediments. Limnol Oceanogr 29:598–608.

Imai KH, Sakaguchi H, Sugio T, Tano T. 1973. On the mechanism of chalcocite oxidation by *Thiobacillus ferrooxidans*. J Ferment Technol 51:865–870.

Ingledew WJ. 1986. Ferrous iron oxidation by *Thiobacillus ferrooxidans*. In: Ehrlich HL, Holmes DS, eds. Biotechnology for the Mining, Metal Refining, and Fossil Fuel Processing Industries. Biotechnol Bioeng Symp 16. New York: Wiley, pp 23–33.

Ivanov VI. 1962. Effect of some factors on iron oxidation by cultures of *Thiobacillus ferrooxidans*. Mikrobiologiya 31:795–799 (Engl transl, pp 645–648).

Ivanov VI, Nagirynyak FI, Stepanov BA. 1961. Bacterial oxidation of sulfide ores. I. Role of *Thiobacillus ferrooxidans* in the oxidation of chalcopyrite and sphalerite. Mikrobiologiya 30:688–692.

Johnson BS, Roberto FF. 1997. Heterotrophic acidophiles and their roles in bioleaching of sulfide minerals. In: Rawlings DE, ed. Biomining: Theory, Microbes and Industrial Processes. Berlin, Germany: Springer-Verlag, pp 259–279.

Jørgensen BB. 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol Oceanogr 22:814–832.

Kee NS, Bloomfield C. 1961. The solution of some minor element oxides by decomposing plant materials. Geochim Cosmochim Acta 24:206–225.

Kelly DP, Wood AP. 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov., and *Thermithiobacillus* gen. nov. Int J Syst Evol Microbiol 50:511–516.

King GM, Howes BL, Dacey JWH. 1985. Short-term end-products of sulfate reduction in a salt marsh: Formation of acid volatile sulfides, elemental sulfur, and pyrite. Geochim Cosmochim Acta 49:1561–1566.

Kleinmann RLP, Crerar DA. 1979. *Thiobacillus ferrooxidans* and the formation of acidity in simulated coal mine environments. Geomicrobiol J 1:373–388.

Klinkhammer GP, Rona P, Greaves M, Elderfeld H. 1985. Hydrothermal manganese plumes in the Mid-Atlantic Ridge Rift Valley. Nature (Lond) 314:727–731.

Labrenz M, Druschel GK, Thomsen-Ebert T, Gilbert B, Welch SA, Kemner KM, Logan GA, Summons RE, De Stasio G, Bond PL, Lai B, Kelly SD, Banfield JF. 2000. Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. Science 290:1744–1747.

Lambert IB, McAndrew J, Jones HE. 1971. Geochemical and bacteriological studies of the cupriferous environment at Pernatty Lagoon, South Australia. Aust Inst Min Met Proc 240:15–23.

Latimer WM, Hildebrand JH. 1942. Reference Book of Inorganic Chemistry. Rev ed. New York: Macmillan.

Lazaroff N. 1983. The exclusion of D_2O from the hydration sphere of $FeSO_4 \cdot 7H_2O$ oxidized by *Thiobacilllus ferrooxidans*. Science 222:1331–1334.

Lazaroff N, Sigal W, Wasserman A. 1982. Iron oxidation and precipitation of ferric hydroxysulfates by resting *Thiobacillus ferrooxidans* cells. Appl Environ Microbiol 43:924–938.

Lazaroff N, Melanson L, Lewis E, Santoro N, Pueschel C. 1985. Scanning electron microscopy and infrared spectroscopy of iron sediments formed by *Thiobacillus ferrooxidans*. Geomicrobiol J 4:231–268.

Leathen WW, Braley SA, McIntyre LD. 1953. The role of bacteria in the formation of acid from certain sulfuritic constituents associated with bituminous coal. II. Ferrous iron oxidizing bacteria. Appl Microbiol 1:65–68.

Lehninger AL. 1975. Biochemistry. 2nd ed. New York: Worth.

Leleu MT, Gulgalski T, Goni J. 1975. Synthèse de wurtzite par voie bacterienne. Mineral Deposita (Berl) 10:323–329.

Love LG. 1962. Biogenic primary sulfide of the Permian Kupferschiefer and marl slate. Econ Geol 57:350–366.

Lovley DR. 2000. Fe(III) and Mn(IV) reduction. In: Lovley DR, ed. Environmental Microbe-Metal Interactions. Washington, DC: ASM Press, pp 3–30.

Lovley DR, Roden EE, Phillips EJP, Woodward JC. 1993. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar Geol 113:41–53.

Lower SK, Hochella MF Jr, Beveridge TJ. 2001. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and α-FeOOH. Science 292:1360–1363.

Lundgren DG, Malouf EE. 1983. Microbial extraction and concentration of metals. Adv Biotechnol Proc 1:223–249.

Lundgren DG, Vestal JR, Tabita FR. 1972. The microbiology of mine drainage pollution. In: Mitchell R, ed. Water Pollution Microbiology. New York: Wiley-Interscience, pp 69–88.

Luther GW III. 1991. Pyrite synthesis via polysulfide compounds. Geochim Cosmochim Acta 55:2839–2849.

Luther GW III, Giblin A, Howarth RW, Ryans RA. 1982. Pyrite and oxidized iron mineral phases formed from pyrite oxidation in salt marsh and estuarine sediments. Geochim Cosmochim Acta 46:2665–2669.

Lyalikova NN. 1960. Participation of *Thiobacillus ferrooxidans* in the oxidation of sulfide ores in pyrite beds of the Middle Ural. Mikrobiologiya 29:382–387.

Mackintosh ME. 1978. Nitrogen fixation by *Thiobacillus ferrooxidans*. J Gen Microbiol 105:215–218.

Magne R, Berthelin J, Dommergues Y. 1973. Solubilisation de l'uranium dans les roches par des bactéries n'appartenant pas au genre *Thiobacillus*. CR Acad Sci (Paris) 276:2625–2628.

Magne R, Berthelin JR, Dommergues Y. 1974. Solubilisation et insolubilisation de l'uranium des granites par des bactéries hétérotroph. In: Formation of Uranium Ore Deposits. Vienna, Austria: Int Atomic Energy Commission, pp 73–88.

Malouf EE, Prater JD. 1961. Role of bacteria in the alteration of sulfide minerals. J Metals 13:353–356.

Marchig V, Grundlach H. 1982. Iron-rich metalliferous sediments on the East Pacific Rise: Prototype of undifferentiated metalliferous sediments on divergent plate boundaries. Earth Planet Sci Lett 58:361–382.

Marnette ECL, Van Breemen N, Hordijk KA, Cappenberg TE. 1993. Pyrite formation in two freshwater systems in the Netherlands. Geochim Cosmochim Acta 57:4165–4177.

McGoran CJM, Duncan DW, Walden CC. 1969. Growth of *Thiobacillus ferrooxidans* on various substrates. Can J Microbiol 15:135–138.

McReady RGL. 1988. Progress in the bacterial leaching of metals in Canada. In: Norris PR, Kelly DP, eds. Biohydrometallurgy. Kew, Surrey, UK: Sci Technol Lett, pp 177–195.

McReady RGL, Gould WD. 1990. Bioleaching of uranium. In: Ehrlich HL, Brierley CL, eds. Microbial Mineral Recovery. New York: McGraw-Hill, pp 107–125.

Miller LP. 1949. Stimulation of hydrogen sulfide production by sulfate-reducing bacteria. Boyce Thompson Inst Contr 15:467–474.

Miller LP. 1950. Formation of metal sulfides through the activities of sulfate reducing bacteria. Boyce Thompson Inst Contr 16:85–89.

Moses CO, Nordstrom DK, Herman JS, Mills AL. 1987. Aqueous pyrite oxidation by dissolved oxygen and by ferric iron. Geochim Cosmochim Acta 51:1561–1571.

Moshuyakova SA, Karavaiko GI, Shchetinina EV. 1971. Role of *Thiobacillus ferrooxidans* in leaching nickel, copper, cobalt, iron, aluminum, magnesium, and calcium from ores of copper-nickel deposits. Mikrobiologiya 40:1100–1107 (Engl transl, pp 959–969).

Mossmann JR, Aplin AC, Curtis CD, Coleman ML. 1991. Geochemistry of inorganic and organic sulfur in organic-rich sediments from the Peru Margin. Geochim Cosmochim Acta 55:3581–3595.

Mottl MJ, Holland HD, Corr RF. 1979. Chemical exchange during hydrothermal alteration of basalt by seawater. II. Experimental results for Fe, Mn, and sulfur species. Geochim Cosmochim Acta 43:869–884.

Mulligan CN, Galvez-Cloutier R. 2000. Bioleaching of copper mining residues by *Aspergillus niger*. Water Sci Technol 41:255–262.

Murr LE, Brierley JA. 1978. The use of large-scale test facilities in studies of the role of microorganisms in commercial leaching operations. In: Murr LE, Torma AE, Brierley JA, eds. Metallurgical Applications of Bacteria. New York: Academic Press, pp 491–520.

Murthy KSN, Natarajan KA. 1992. The role of surface attachment of *Thiobacillus ferrooxidans* on the oxidation of pyrite. Miner Metall Process, February: pp 20–24.

Mustin C, Berthelin J, Marion P, de Donato P. 1992. Corrosion and electrochemical oxidation of a pyrite by *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58:1175–1182.

Mustin C, de Donato P, Berthelin J. 1993. Surface oxidized species, a key factor in the study of bioleaching processes. In: Torma AE, Wey JE, Lakshmanan VI, eds. Biohydrometallurgical Technologies, Vol 1, Bioleaching Process. Warrendale, PA: Miner, Metals Mater Soc, pp 175–184.

Myers CR, Myers JM. 1997. Outer membrane cytochromes of *Shewanella putrefaciens* MR-1: Spectral analysis, and purification of the 83-kDa c-type cytochrome. Biochim Biophys Acta 1326:307–318.

Myers JM, Myers CR. 1998. Isolation and sequence of omcA, a gene encoding dodecaheme outer membrane cytochrome c of *Shewanella putrefaciens* MR-1, and detection of omcA homologs in other strains of *S. putrefaciens*. Biochim Biophys Acta 1373:237–251.

Myers JM, Myers CR. 2001. Role of outer membrane cytochromes OmcA and OmcB of *Shewanella putrefaciens* MR-1 in reduction of manganese dioxide. Appl Environ Microbiol 67:260–269.

Nedwell DB, Banat IM. 1981. Hydrogen as an electron donor for sulfate-reducing bacteria in slurries of salt marsh sediment. Microb Ecol 7:305–313.

Nielsen AM, Beck JV. 1972. Chalcocite oxidation and coupled carbon dioxide fixation by *Thiobacillus ferrooxidans*. Science 175:1124–1126.

Nordstrom DK, Southam G. 1997. Geomicrobiology of sulfide mineral oxidation. In: Banfield JF, Nealson KH, eds. Geomicrobiology: Interactions Between Microbes and Minerals. Rev Mineral 35:361–390.

Norman PG, Snyman CP. 1988. The biological and chemical leaching of an auriferous pyrite/arsenopyrite flotation concentrate: A microscopic examination. Geomicrobiol J 6:1–10.

Norris PR. 1990. Acidophilic bacteria and their activity in mineral sulfide oxidation. In: Ehrlich HL, Brierley CL, eds. Microbial Mineral Recovery. New York: McGraw-Hill, pp 3–27.

Norris PR. 1997. Thermophiles and bioleaching. In: Rawlings DE, ed. Biomining: Theory, Microbes and Industrial Processes. Berlin, Germany: Springer-Verlag, pp 247–258.

Parès Y. 1964a. Intervention des bactéries dans la solubilisation du cuivre. Ann Inst Pasteur (Paris) 107:132–135.

Parès Y. 1964b. Action de *Serratia marcescens* dans le cycle biologique des métaux. Ann Inst Pasteur (Paris) 107:136–141.

Parès Y. 1964c. Action d'*Agrobacterium tumefaciens* dans la mise en solution de l'or. Ann Inst Pasteur (Paris) 107:141–143.

Pinka J. 1991. Bacterial oxidation of pyrite and pyrrhotite. Erzmetall 44:571–573.
Pistorio M, Curutchet G, Donati R, Tedesco P. 1994. Direct zinc sulfide bioleaching by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Biotechnol Lett 16:419–424.
Pivovarova TA, Markosyan GE, Karavaiko GI. 1981. Morphogenesis and fine structure of *Leptospirillum ferrooxidans*. Mikrobiologiya 50:482–486 (Engl transl, pp 339–344).
Pogliani C, Curutchet G, Donati E, Tedesco PH. 1990. A need for direct contact with particle surfaces in the bacterial oxidation of covellite in the absence of a chemical lixiviant. Biotechnol Lett 12:515–518.
Powell TG, MacQueen RW. 1984. Precipitation of sulfide ores and organic matter: Sulfate reduction at Pine Point, Canada. Science 224:63–66.
Pronk JT, Meulenberg R, Hazeu W, Bos, P, Kuenen JG. 1990. Oxidation of reduced inorganic sulfur compounds by acidophilic thiobacilli. FEMS Microbiol Rev 75:293–306.
Rawlings DE. 1997a. Biomining: Theory, Microbes and Industrial Processes. Berlin, Germany: Springer-Verlag.
Rawlings DE. 1997b. Mesophilic, autotrophic bioleaching bacteria: Description, physiology and role. In: Rawlings DE, ed. Biomining: Theory, Microbes and Industrial Processes. Berlin, Germany: Springer-Verlag, pp 229–245.
Rawlings DE, Tributsch H, Hansford GS. 1999. Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology (Reading) 145:5–13.
Razzell WE, Trussell PC. 1963. Microbiological leaching of metallic sulfides. Appl Microbiol 11:105–110.
Rickard DT. 1973. Limiting conditions for synsedimentary sulfide ore formation. Econ Geol 68:605–617.
Rickard PAD, Vanselow DG. 1978. Investigations into the kinetics and stoichiometry of bacterial oxidation of covellite (CuS) using a polarographic oxygen probe. Can J Microbiol 24:998–1003.
Rodriguez-Leiva M, Tributsch H. 1988. Morphology of bacterial leaching patterns by *Thiobacillus ferrooxidans* on synthetic pyrite. Arch Microbiol 149:401–405.
Roy AB, Trudinger PA. 1970. The Biochemistry of Inorganic Compounds of Sulphur. Cambridge, UK: Cambridge Univ. Press.
Sand W, Rohde K, Sabotke B, Zenneck C. 1992. Evaluation of *Leptospirillum ferrooxidans* for leaching. Appl Environ Microbiol 58:85–92.
Sand W, Gehrke T, Hallmann R, Rohde K, Sabotke B, Wentzien S. 1993. In-situ bioleaching of metal sulfides: The importance of *Leptospirillum ferrooxidans*. In: Torma AE, Wey JE, Lakshmanan VL. Biolydrometallurgical Technologies, Vol I, Warrendale, PA: The Minerals, Metals and Materials Society, pp 15–27.
Sand W, Gehrke T, Hallmann, R, Schippers A. 1995. Sulfur chemistry, biofilm, and the (in)direct attack mechanism: A critical evaluation of bacterial leaching. Appl Microbiol Biotechnol 43:961–966.
Sand, W, Gehrke T, Jozsa P-G, Schippers A. 1997. Novel mechanism for bioleaching of metal sulfides. In: Biotechnology Comes of Age. Int Biohydrometall Symp IBS97 BIOMINE 97. Conf Procs. Glenside, South Australia: Australian Mineral Foundation, pp QP2.1–QP2.10.

Schippers A, Sand W. 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Appl Environ Microbiol 65:319–321.

Schoonen MAS, Barnes HL. 1991a. Reactions forming pyrite and marcasite from solution. I. Nucleation of FeS_2 below 100°C. Geochim Cosmochim Acta 55:1495–1504.

Schoonen MAS, Barnes HL. 1991b. Reactions forming pyrite and marcasite from solution. II. Via FeS precursors below 100°C. Geochim Cosmochim Acta 55:1505–1514.

Schrenk MO, Edwards KJ, Goodman RM, Hamers RJ, Banfield JF. 1998. Distribution of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*: Implications for generation of acid mine drainage. Science 279:1519–1522.

Segerer A, Neuner A, Kristjansson JK, Stetter KO. 1986. *Acidianus infernus* gen. nov. sp. nov., and *Acidianus brierleyi* comb. nov.: Facultative aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaebacteria. Arch Microbiol 36:559–564.

Serkies J, Oberc J, Idzikowski A. 1967. The geochemical bearings of the genesis of Zechstein copper deposits in southwest Poland as exemplified by the studies on the Zechstein of the Leszczyna syncline. Chem Geol 2:217–232.

Seyfried WE Jr, Mottl MJ. 1982. Hydrothermal alteration of basalt by seawater under seawater-dominated conditions. Geochim Cosmochim Acta 46:985–1002.

Shanks WC III, Bischoff JL, Rosenauer RJ. 1981. Seawater sulfate reduction and sulfur isotope fractionation in basaltic systems: Interaction of seawater with fayalite and magnetite at 200–350°C. Geochim Cosmochim Acta 45:1977–1981.

Shrihari RK, Ghandi KS, Natarajan KA. 1991. Role of cell attachment in leaching chalcopyrite mineral by *Thiobacillus ferrooxidans*. Appl Microbiol Biotechnol 36:278–282.

Silver M, Torma AE. 1974. Oxidation of metal sulfides by *Thiobacillus ferrooxidans* grown on different substrates. Can J Microbiol 20:141–147.

Silverman MP, Ehrlich HL. 1964. Microbial formation and degradation of minerals. Adv Appl Microbiol 6:153–206.

Silverman MP, Rogoff MH, Wender I. 1961. Bacterial oxidation of pyritic materials in coal. Appl Microbiol 9:491–496.

Singer PC, Stumm W. 1970. Acidic mine drainage: The rate-determining step. Science 167:1121–1123.

Stanton RL. 1972. Sulfides in sediments. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser, Vol IVA. New York: Van Nostrand Reinhold, pp 1134–1141.

Stevens CJ, Dugan PR, Tuovinen OH. 1986. Acetylene reduction (nitrogen fixation) by *Thiobacillus ferrooxidans*. Biotechnol Appl Biochem 8:351–359.

Strahler AN. 1977. Principles of Physical Geology. New York: Harper & Row.

Styrt MM, Brackmann AJ, Holland HD, Clark BC, Pisutha-Arnold V, Eldridge CS, Ohmoto H. 1981. The mineralogy and the isotopic composition of sulfur in hydrothermal sulfide/sulfate deposits on the East Pacific Rise, 21°N latitude. Earth Planet Sci Lett 53:382–390.

Sugio T, Tanijiri S, Fukuda K, Yamargo K, Inagaki K, Tano T. 1987. Utilization of amino acids as a sole source of nitrogen by obligate chemoautotroph *Thiobacillus ferrooxidans*. Agric Biol Chem 51:2229–2236.

Sullivan JD. 1930. Chemistry of leaching covellite. Tech Paper 487. Washington, DC: US, Dept of Commerce, Bur Mines.
Sutton JA, Corrick JD. 1963. Microbial leaching of copper minerals. Mining Eng 15:37–40.
Sutton JA, Corrick JD. 1964. Bacteria in mining and metallurgy: Leaching selected ores and minerals; experiments with *Thiobacilllus ferrooxidans*. Rept Invest RI 5839. Washington, DC: Bur Mines, US Dept of the Interior.
Temple KL. 1964. Syngenesis of sulfide ores. An explanation of biochemical aspects. Econ Geol 59:1473–1491.
Temple KL, LeRoux N. 1964. Syngenesis of sulfide ores: Desorption of adsorbed metal ions and the precipitation as sulfides. Econ Geol 59:647–655.
Thode-Andersen S, Jørgensen BB. 1989. Sulfate reduction and the formation of ^{35}S-labeled FeS, FeS_2, and S^0 in coastal marine sediments. Limnol Oceanogr 34:793–806.
Tittley SR. 1981. Porphyry copper. Am Sci 69:632–638.
Torma AE. 1971. Microbial oxidation of synthetic cobalt, nickel and zinc sulfides by *Thiobacillus ferrooxidans*. Rev Can Biol 30:209–216.
Torma AE. 1978. Oxidation of gallium sulfides by *Thiobacillus ferrooxidans*. Can J Microbiol 24:888–891.
Torma AE, Gabra GG. 1977. Oxidation of stibnite by *Thiobacillus ferrooxidans*. Antonie v Leeuwenhoek 43:1–6.
Tributsch H. 1976. The oxidative disintegration of sulfide crystals by *Thiobacilllus ferrooxidans*. Naturwissenschaften 63:88.
Tributsch H. 1999. Direct versus indirect bioleaching. In: Amils R, Ballester A, eds. Biohydrometallurgy and the Environment Toward the Mining of the 21st Century, Part A. Amsterdam: Elsevier, pp 51–60.
Tuovinen OH, Niemelä SI, Gyllenberg HG. 1971. Tolerance of *Thiobacillus ferrooxidans* to some metals. Antonie v Leeuwenhoek 37:489–496.
Wadden D, Gallant A. 1985. The in-place leaching of uranium at Denison Mines. Can Metall Quart 24:127–134.
Walsh F, Mitchell R. 1972a. An acid-tolerant iron-oxidizing *Metallogenium*. J Gen Microbiol 72:369–376.
Walsh F, Mitchell R. 1972b. The pH-dependent succession of iron bacteria. Environ Sci Technol 6:809–812.
Weast RC, Astle MJ. 1982. CRC Handbook of Chemistry and Physics. 63rd ed. Boca Raton, FL: CRC Press.
Wenberg GM, Erbisch FH, Volin M. 1971. Leaching of copper by fungi. Trans Soc Min Eng AIME 250:207–212.
Westrich JT, Berner RA. 1984. The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. Limnol Oceanogr 29:236–249.
Zajic JE. 1969. Microbial Biogeochemistry. New York: Academic Press.
Zimmerley SR, Wilson DG, Prater JD. 1958. Cyclic leaching process employing iron oxidizing bacteria. US Patent 2,829,964.

20

Geomicrobiology of Selenium and Tellurium

20.1 OCCURRENCE IN THE EARTH'S CRUST

The elements selenium and tellurium, like sulfur, belong to group VI of the periodic table. All three have some properties in common, but selenium and tellurium, especially the latter, have some metallic attributes, unlike sulfur. Selenium and tellurium are much less abundant than sulfur in the Earth's crust. Selenium amounts to only 0.05–0.14 ppm (Rapp, 1972, p. 1080) and tellurium to 10^{-5}–10^{-2} ppm (Lansche, 1965). Both are associated with metal sulfides in nature and occur in distinct minerals [e.g., ferroselite ($FeSe_2$), challomenite ($CuSeO_3 \cdot 2H_2O$), hessite (Ag_2Te), and tetradymite (Bi_2Te_2S)]. Selenium occurs in small amounts in various soils in concentrations in the range of 0.01–100 ppm. High concentrations are associated with arid, alkaline soils that contain some free $CaCO_3$ (Rosenfeld and Beath, 1964).

20.2 BIOLOGICAL IMPORTANCE

Some plants, such as *Astragalus* spp. and *Stanleya*, can accumulate large amounts of selenium in the form of organic selenium compounds. However, not all forms of selenium in soil are available for assimilation by these plants.

Selenium is required nutritionally as a trace element by at least some microorganisms, plants, and animals, including human beings (Stadtman, 1974;

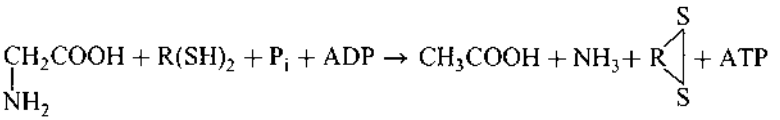
Miller and Neathery, 1977; Combs and Scott, 1977; Patrick, 1978; Mertz, 1981). It has been found to be an essential component of the enzyme glutathione peroxidase in mammalian red blood corpuscles (Rotruck et al., 1973). The enzyme catalyzes the reaction

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \tag{20.1}$$

Selenium has also been found to be essential together with molybdenum in the structure of formate dehydrogenase in the bacteria *Escherichia coli*, *Clostridum thermoaceticum*, *C. sticklandii*, and *Methanococcus vannielii* among others (Pinsent, 1954; Lester and DeMoss, 1971; Shum and Murphy, 1972; Andreesen and Ljungdahl, 1973; Enoch and Lester, 1972; Stadtman, 1974) and with tungsten in the formate dehydrogenase of *C. thermoaceticum* when grown in the presence of tungsten instead of molybdenum (Yamamoto et al., 1983). The enzyme catalyzes the reaction

$$HCOOH + NAD^+ \rightarrow CO_2 + NADH + H^+ \tag{20.2}$$

Selenium has also been found to be essential to protein A of glycine reductase in Clostridia (Stadtman, 1974), an enzyme that catalyzes the reaction



(20.3)

For this reason, *Clostridium purinolyticum* exhibits an absolute requirement for selenium in its growth medium for fermentation of glycine (Duerre and Andreesen, 1982).

No biological requirement for tellurium has been observed up to now.

20.3 TOXICITY OF SELENIUM AND TELLURIUM

Both selenium and tellurium are toxic when present in excess, but the minimum toxic doses vary depending on the organism. As mentioned in Section 20.2, some plants accumulate selenium to the extent of 1.5–2 g kg^{-1} dry weight of tissue (Stadtman, 1974). They usually grow in arid environments with unusually high concentrations of selenium in the soil. In the Kesterson National Wildlife Refuge in California, where extensive selenium intoxication of wild animals has been observed, selenate concentrations of 1.8–18 μM (0.14–1.4 ppm) have been reported, contrasted with normal concentrations of ~1.3–21.2 nM in the San Joaquin River, ~0.4–1.3 nM in the Sacramento River, and < 0.2 nM in San Francisco Bay, all located in California (Zehr and Oremland, 1987). These normal

concentrations are below minimum inhibitory concentrations (MICs) of selenate [Se(VI)] for three selenium-sensitive strains of bacteria from the same general area in California. Their MICs were found to range from 0.78 to 1.56 mM for selenate and from 1.56 to 25 mM for selenite [Se(IV)]. Selenium-resistant bacteria from the Kesterson National Wildlife Refuge exhibited MICs of 50 to <200 mM selenite (Burton et al., 1987). By contrast, both selenium-resistant and selenium-sensitive organisms from these same sites in California exhibited MICs for tellurate in the range of 0.03–1 mM and for tellurite in the range of 0.03–4 mM (Burton et al., 1987).

Selenium and tellurium resistance appear to be regulated by different genes. In *Escherichia coli*, tellurium resistance appears to be mediated by the arsenical ATPase efflux pump. The genetic determinants for this pump reside on resistance plasmid R773 (Turner et al., 1992). Higher forms of life appear to be relatively more sensitive to Se than bacteria, even though they require Se as a nutritional trace element. Biochemically, Se toxicity appears to be the result of superoxide or H_2O_2 production in excess of antioxidant production by a cell. A similar mechanism may be the basis for Te toxicity (see references 31, 35, and 41 cited by Guzzo and Dubow, 2000).

20.4 BIO-OXIDATION OF REDUCED FORMS OF SELENIUM

Some inorganic forms of selenium have been reported to be oxidizable by microorganisms. *Micrococcus selenicus* isolated from mud (Breed et al., 1948), a rod-shaped bacterium isolated from soil and thought to be autotrophic (Lipman and Waksman, 1923), and a purple bacterium (Sapozhnikov, 1937) were observed to oxidize Se^0 to SeO_4^{2-}. A strain of *Bacillus memegaterium* from top soil in a river alluvium was found to oxidize Se^0 to SeO_3^{2-} and traces of SeO_4^{2-}. Red selenium was more readily attacked than gray selenium (Sarathchandra and Watkinson, 1981). Dowdle and Oremland (1998) observed elemental selenium oxidation in soil slurries that was inhibited by autoclaving the slurry or by addition of formalin, azide, 2,4-dinitrophenol, or the antibiotics chloramphenicol + tetracycline or cycloheximide + nystatin. Se^0 oxidation in the slurries was enhanced by addition of sulfide, acetate, or glucose, suggesting that sulfur-oxidizing autotrophs and heterotrophs were involved in the oxidation.

Thiobacillus ferrooxidans (now *Acidithiobacillus ferrooxidans*) has been shown to oxidize copper selenide (CuSe) to cupric copper (Cu^{2+}) and elemental selenium (Se^0) (Torma and Habashi, 1972). The reaction may be written

$$CuSe + 2H^+ + 0.5O_2 \rightarrow Cu^{2+} + Se^0 + H_2O \qquad (20.4)$$

20.5 BIOREDUCTION OF OXIDIZED SELENIUM COMPOUNDS

Various inorganic selenium compounds have been found to be reduced anaerobically by some microorganisms. Crude cell extract of *Micrococcus lactilyticus* (also known as *Veillonella lactilyticus*) has been shown to reduce selenite but not selenate to Se^0, and Se^0 to HSe^-. The reductant was hydrogen (Woolfolk and Whiteley, 1962). Cell extracts from strains *D. desulfuricans* and *C. pasteurianum* were also found to reduce selenite with hydrogen. The enzyme hydrogenase mediated electron transfer from hydrogen in these reactions (Woolfolk and Whiteley, 1962). A variety of other bacteria, actinomycetes, and fungi have been shown to reduce selenate and selenite to Se^0 (Bautista and Alexander, 1972; Lortie et al., 1992; Tomei et al., 1992; Zalokar, 1953; Stolz and Oremland, 1999). The bacteria include *Pseudomonas stutzeri*, *Wolinella succinogenes*, and *Micrococcus* sp. *Thiobacillus* (now *Acidithiobacillus*) *ferrooxidans* is able to reduce Se^0 (red form) to H_2Se anaerobically, albeit in small quantities (Bacon and Ingledew, 1989).

A relatively recently discovered bacterium, *Thauera selenatis* can grow anaerobically with selenate or nitrate as terminal electron acceptor (Macy et al., 1993; Rech and Macy, 1992). In the absence of nitrate it reduces selenate to selenite (DeMoll-Decker and Macy, 1993). The reductases for selenate and nitrate in this organism are distinct enzymes with different pH optima. Thus in contrast to the response of a selenate-reducing enrichment culture (Steinberg et al., 1992), nitrate does not inhibit selenate reduction by *T. selenatis*. Indeed, when present together, both selenate and nitrate are reduced simultaneously, with selenate being reduced to elemental selenium (DeMoll-Decker and Macy, 1993). The selenate reductase in this organism, which catalyzes the reduction of selenate to selenite, is found in its periplasm, whereas its nitrate reductase, which catalyzes the reduction of nitrate to nitrite, is found in its cytoplasmic membrane (Rech and Macy, 1992). Selenate reductase is a metalloprotein containing Mo, Fe, acid-labile sulfur, and a cytochrome b subunit (Schroeder et al., 1997). Nitrite reductase is found in the periplasm of *T. selenatis* and plays a role in selenite reduction, besides catalyzing nitrite reduction (DeMoll-Decker and Macy, 1993). This helps to explain why *T. selenatis* produces elemental selenium in the presence of nitrate but selenite in its absence. Selenite does not support growth of *T. selenatis* (DeMoll-Decker and Macy, 1993).

A selenite reductase enzyme has been obtained from the fungus *Candida albicans* (Falcone and Nickerson, 1963; Nickerson and Falcone, 1963). It reduces selenite to Se^0. A characterization of the enzyme has shown that it requires a quinone, a thiol compound (e.g., glutathione), a pyridine nucleotide (NADP), and an electron donor (e.g., glucose 6-phosphate) for activity. Electron transfer between NADP and quinone is probably mediated by flavin mononucleotide in

this system. It is possible that this enzyme is part of an assimilatory SeO_4^{2-} and/or SeO_3^{2-} reductase system. How this enzyme compares with that in *T. selenatis* remains to be established.

Sulfurospirillum barnesii (formerly *Geospirillum barnesii*, also called strain SES-3) (Oremland et al., 1994; Stolz et al., 1999) is another bacterium that can reduce selenate to elemental selenium. Cells of this organism grew with lactate as carbon and energy source and selenate as terminal electron acceptor, which was reduced to selenite. As with *Thauera selenatis*, resting cells of *S. barnesii* but not growing cells were able to reduce selenite to Se^0 (Oremland et al., 1994). One important difference between *S. barnesii* and *T. selenatis* is that *S. barnesii* is able to use a much wider range of reducible anions as terminal electron acceptors than *T. selenatis* (Stolz and Oremland, 1999). *S. barnesii* can reduce selenate and nitrate simultaneously whether pregrown on selenate or nitrate, consistent with the observation that selenate reductase is constitutive in this organism (Oremland et al., 1999).

Two newly discovered selenate reducers, both gram-positive bacteria, are *Bacillus arsenicoselenatis* and *Bacillus selenitireducens* (Switzer Blum et al., 1998). The first forms spores but the second does not. Both were isolated from anoxic muds from Mono Lake, California, which is alkaline, hypersaline, and arsenic-rich. *B. arsenicoselenatis* reduces selenate to selenite whereas *B. selenitireducens* reduces selenite to elemental selenium. Both strains can reduce arsenate as well as selenate. *B. arsenicoselenatis* can also reduce selenite and nitrate simultaneously, but its selenate reductase is not constitutive in nitrate-grown cells (Oremland et al., 1999). Therefore, in order for this organism to reduce selenate and nitrate simultaneously, it has to be grown in the presence of a mixture of the two electron acceptors.

A moderately halophilic selenate reducer was isolated from Dead Sea (Israel) sediment. It reduced selenate to selenite and elemental selenium. It is a gram-negative organism and has been named *Selenihalanaerobacter shriftii* (Switzer Blum et al., 2001). When it respires on glycerol or glucose, it forms acetate plus CO_2. Nitrate and trimethylamine *N*-oxide could serve as alternative electron acceptors, but reduced forms of sulfur, nitrite, arsenate, fumarate, or dimethylsulfoxide could not.

All previously mentioned selenate- and selenite-reducing bacteria belong in the domain Bacteria. Recently a hyperthermophilic member of the domain Archaea capable of respiring organotrophically on selenate was isolated from a hot spring near Naples, Italy (Huber et al., 2000) (see also Chap. 13). Its name is *Pyrobaculum arsenaticum*. It reduces selenate to elemental selenium. Previously isolated *Pyrobaculum aerophilum* (Völkl et al., 1993) was found capable of respiring organotrophically on selenate and selenite and autotrophically on selenate with H_2 as electron donor (Huber et al., 2000). Elemental selenium was the reduction product.

In most studies of bacterial reduction of selenate and selenite, elemental selenium (red form) is usually found to be a major, if not the only, product. This is noteworthy because sulfate and sulfite cannot be directly reduced to S^0 but are reduced to H_2S without intermediate formation of S^0. Yet selenium and sulfur are members of the same chemical family. The implication is that enzymatic mechanisms of reduction for oxidized forms of these two elements are different. To date, none of the true selenate respirers have been found capable of sulfate respiration, which could be related to the significantly higher energy yield in selenate respiration ($\Delta G'$ -15.53 kcal mol^{-1} e) than in sulfate respiration ($\Delta G'$ -0.10 kcal mol^{-1} e^{-1}) (Newman et al., 1998). It must be noted, however, that *Desulfovibrio desulfuricans* subsp. *aestuarii* has been found to reduce nanomolar but not millimolar quantities of selenate to selenide (Zehr and Oremland, 1987). Sulfate inhibited the reduction of selenate, suggesting but not proving that the mechanism of sulfate and selenate reduction in this case may be a common one. As Zehr and Oremland (1987) pointed out, when sulfate is being reduced to H_2S in the absence of selenate, some of the H_2S formed may chemically reduce biogenically formed selenite to Se^0. They found that in nature the sulfate reducer can reduce selenate only if the ambient sulfate concentration is below 4 mM.

Whereas selenate and/or selenite reduction by the previously described organism resulted in extracellular deposition of Se^0, intracellular deposition of Se^0 has been observed with some other organisms. *Chromatium vinosum* can deposit Se^0 intracellularly as a result of an interaction of H_2Se, which is produced by *Desulfovibrio desulfuricans* in selenate reduction in co-culture with *C. vinosum*. The Se^0 is stored in the form of globules in the *C. vinosum* cells (Nelson et al., 1996). *Rhodobacter spheroides* deposited red Se^0 produced in the reduction of selenate and selenite in or on its cells (Van Fleet-Stadler et al., 2000). *Ralstonia metallidurans* CH34 can reduce selenite to red Se^0, which it stores in its cytoplasm and occasionally in its periplasm (Roux et al., 2001).

Other Products of Selenate and Selenite Reduction

In *Escherichia coli*, a significant portion of selenite reduced during glucose metabolism is deposited as Se^0 on its cell membrane but not in its cytoplasm (Gerrard et al., 1974), and another portion is incorporated as selenide in organic compounds such as selenomethionine (Ahluwalia et al., 1968). Some soil microbes reduce selenate or selenite to dimethyl selenide [$(CH_3)_2Se$] at elevated selenium concentrations (Kovalskii et al., 1968; Fleming and Alexander, 1972; Alexander, 1977; Doran and Alexander, 1977). Other volatile selenium compounds may also be formed, their relative quantities depending on reaction conditions (e.g., Reamer and Zoller, 1980). These compounds include dimethyl diselenide [$(CH_3)_2Se_2$] and dimethyl selenone [$(CH_3)_2SeO_2$].

Among microorganisms, some fungi have been found to be effective in forming methylated selenium compounds (Barkes and Fleming, 1974). *Alternaria alternata* isolated from seleniferous water from a sample series collected from evaporation ponds at the Kesterson Reservoir, Lost Hills, and Peck Ranch in California formed dimethylselenide more rapidly from selenate and selenite than from selenium sulfide (SeS_2) or various organic Se compounds. Methionine, a known biochemical methyl donor, and methylcobalamin, a known methyl carrier in biochemical transmethylation, stimulated dimethylselenide formation by the fungus (Thompson-Eagle et al., 1989). Crude cell extracts and a supernatant fraction from the fungus *Pichia guillermondi* after centrifugation at $144{,}000 \times g$ reduced selenite but not selenate (Bautista and Alexander, 1972). In a mechanism proposed by Reamer and Zoller (1980), all methylated forms of selenium arise by methylation of selenite and subsequent reductions and, where needed further, methylation. Dimethylselenone is viewed as a precursor of dimethylselenide, whereas methylselenide $(CH_3)SeH$ and/or $(CH_3)SeOH$ is viewed as a precursor of dimethyldiselenide.

Selenium Reduction in the Environment

Bacterial reduction of selenate and selenite has been detected in situ, especially in environments with significant soluble selenium. In the Kesterson National Wildlife Refuge, California, Maiers et al. (1988) reported that 4% of water samples, 92% of sediment samples, and 100% of the soil samples they collected exhibited microbial selenium reduction. Of 100 mg selenate per liter, up to 75% was reduced to red Se^0, the rest to selenite. In the interstitial water of core samples from a wastewater evaporation pond in Fresno, California, selenate removal was stimulated by H_2 and by the addition of acetate and inhibited by O_2, NO_3^-, MnO_2, CrO_4^{2-}, and WO_4^{2-}, but not by the addition of SO_4^{2-}, MoO_4^{2-}, or FeOOH (Oremland et al., 1989). At other sites in California and also in Nevada, Steinberg and Oremland (1990) found measurable selenate-reducing activity in surficial sediment samples from bodies of freshwater to salinities of 250 g L^{-1} but not 320 g L^{-1}. Nitrate, nitrite, molybdate, and tungstate added separately to samples from agricultural drains were inhibitory to different extents. Sulfate partially inhibited the reduction of selenate in a sample from a freshwater site but not in one from a site with water having a salinity of 60 g L^{-1}. These differences are likely reflections of differences in the types of selenate reducers in the different samples and therefore of differences in mechanisms of selenate reduction. Additional studies in the agricultural drainage region of western Nevada revealed a selenate turnover rate of 0.04–1.8 hr^{-1} at ambient Se oxyanion concentrations (13–455 nM). Rates of removal of selenium oxyanions ranged from 14 to 155 µmol m^{-2} day^{-1} (Oremland et al., 1991). Formation of elemental Se has a potential for selenium immobilization in soil and

sediment under anaerobic conditions. Owing to the possibility of Se^0 reoxidation under aerobic conditions, remobilization may occur. However, such reoxidation has been found to be a slow process compared to microbial selenate reduction (Dowdle and Oremland, 1998).

Methylation of selenium in aquatic environments has also been observed (e.g., Chau et al., 1976; Frankenberger and Karlson, 1992, 1995). This activity has a potential for Se removal from polluted soils and waters.

Ecologically, anaerobic reduction of selenate and selenite to selenium represents a respiratory, energy-conserving process in some microorganisms and serves to detoxify the immediate environment for all organisms as long as anaerobic conditions are maintained. Selenium volatilization serves as a permanent detoxification process in water, soils, and sediments, and can occur aerobically, although in at least one instance it was more effective anaerobically (Frankenberger and Karlson, 1995).

20.6 SELENIUM CYCLE

The existence of a selenium cycle in nature was suggested by Shrift (1964). However, some of the details of this cycle are still obscure. The ultimate source of selenium must be igneous rocks, but whether microbes play a role in mobilizing the selenium from selenium-containing minerals is unknown. Similarly, little is known about the role that microbes may play in mobilizing selenium in soil and sediment. Such activity, when it occurs, is of great importance in understanding and controlling selenium pollution, as has occurred, for instance, in the Kesterson National Wildlife Refuge in California. The source of selenium in that case appears to be the drainage of irrigation water applied to farmland in the San Joaquin Valley. The irrigation water leached selenium from the soil. This drainage has been collecting in the wildlife refuge. Different processes in selenium cycling in wetlands include redox reactions involving selenium, methylation and volatilization of selenium, organic and inorganic complexation of selenium, precipitation and dissolution of Se-containing minerals, and sorption and desorption of ionic species of selenium (Masscheleyn and Patrick, 1993). Known biochemical steps of a selenium cycle are shown in Fig. 20.1.

20.7 BIO-OXIDATION OF REDUCED FORMS OF TELLURIUM

Microbial oxidation of reduced forms of tellurium has so far not been reported. This may mean that this process does not occur in nature, but it is more likely that so far it has not been sought by investigators. Its geomicrobial importance is

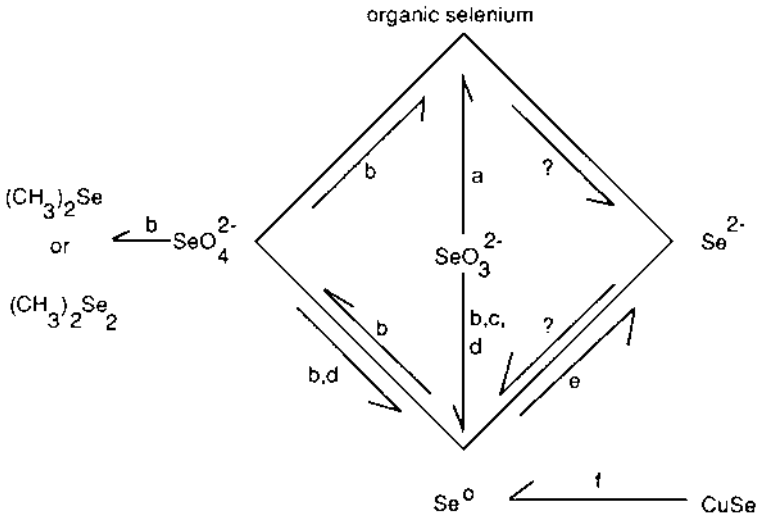


FIG. 20.1 The selenium cycle. (a) *Escherichia coli*; (b) bacteria; (c) actinomycetes; (d) fungi; (e) *M. lactilyticus*; (f) *T. ferrooxidans*. (See also Doran and Alexander, 1977.)

likely to be limited, because the occurrence of tellurium is much rarer than that of selenium (see Sec. 20.1).

20.8 BIOREDUCTION OF OXIDIZED FORMS OF TELLURIUM

Microbial reduction of tellurates and tellurites to Te^0 or $(CH_3)_2Te$ has been reported (Bautista and Alexander, 1972; Silverman and Ehrlich, 1964; Nagai, 1965; Woolfolk and Whiteley, 1962; Trutko et al., 2000). Trutko et al. (2000) presented evidence that the respiratory chain was involved in tellurite reduction by some gram-negative bacteria. The tellurite was reduced to tellurium crystallites, which appeared in the periplasmic space or on the outer or inner surface of the plasma membrane. The makeup of the respiratory chains differed to some extent among the different bacterial cultures tested.

The fungus *Penicillium* sp. has been found to produce $(CH_3)_2Te$ from several inorganic tellurium compounds, provided only that reducible selenium compounds were also present (Fleming and Alexander, 1972). The amount of dialkyltelluride formed was related to the relative concentrations of Se and Te in the medium. Microbial reduction of oxidized forms of tellurium may represent detoxification reactions rather than a form of respiration, but this needs further investigation.

20.9 SUMMARY

Selenium, although a very toxic element, is nutritionally required by some bacteria, plants, and animals. Microorganisms have been described that can oxidize reduced selenium compounds. At least one, *Thiobacillus ferrooxidans*, can use selenide in the form of CuSe as a sole source of energy, oxidizing the compound to elemental selenium (Se^0) and Cu^{2+}. Oxidized forms of inorganic selenium compounds can be reduced by microorganisms, including bacteria and fungi. Selenate and selenite may be reduced to one or more of the following: Se^0, H_2Se, dimethyl selenide [$(CH_3)_2Se$], dimethyl diselenide [$(CH_3)_2Se_2$], and dimethyl selenone [$(CH_3)_2SeO_2$]. The reductions are enzymatic. The microbial reactions contribute to a selenium cycle in nature. Microbial selenate and selenite reduction to elemental selenium in soil and sediment is a form of selenium immobilization that is potentially reversible. Microbial selenate and selenite reduction to volatile forms of selenium in soil, sediment, and water columns of bodies of water is a form of selenium removal that is permanent.

Tellurium occurs in such low concentrations in nature that it does not seem geomicrobiologically important. Nevertheless, microbial reduction of tellurate and tellurite to elemental tellurium (Te^0) and dimethyltelluride [$(CH_3)_2Te$] has been observed. Microbial oxidation of tellurides has not been reported.

REFERENCES

Ahluwalia GS, Saxena YR, Williams HH. 1968. Quantitation studies on selenite metabolism in *Escherichia coli*. Arch Biochem Biophys 124:79–84.

Alexander M. 1977. Introduction to Soil Microbiology. 2nd ed. New York: Wiley.

Andreesen JR, Ljungdahl LG. 1973. Formate dehydrogenase of *Clostridium thermoaceticum*: Incorporation of selenium-75, and the effects of selenite, molybdate, and tungstate on the enzyme. J Bacteriol 116:869–873.

Bacon M, Ingledew WJ. 1989. The reductive reactions of *Thiobacillus ferrooxidans* on sulfur and selenium. FEMS Microbiol Lett 58:189–194.

Barkes L, Fleming RW. 1974. Production of dimethylselenide gas from inorganic selenium by eleven fungi. Bull Environ Contam Toxicol 12:308–311.

Bautista EM, Alexander M. 1972. Reduction of inorganic compounds by soil microorganisms. Soil Sci Soc Am Proc 36:918–920.

Breed RS, Murray EGD, Smith NR. 1948. Bergey's Manual of Determinative Bacteriology. 6th ed. Baltimore, MD: Williams & Wilkins.

Burton GA Jr, Giddings TH, DeBrine P, Fall R. 1987. High incidence of selenite-resistant bacteria from a site polluted with selenium. Appl Environ Microbiol 53:185–188.

Chau YK, Wong PTS, Silverberg BA, Luxon PL, Bengert GA. 1976. Methylation of selenium in the aquatic environment. Science 192:1130–1131.

Combs GF Jr, Scott ML. 1977. Nutritional interrelationships of vitamin E and selenium. BioScience 27:467–473.

DeMoll-Decker H, Macy JM. 1993. The periplasmic nitrite reductase of *Thauera selenatis* may catalyze the reduction of selenite to elemental selenium. Arch Microbiol 160:241–247.

Doran JW, Alexander M. 1977. Microbial transformation of selenium. Appl Environ Microbiol 33:31–37.

Dowdle PR, Oremland RS. 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. Environ Sci Technol 32:3749–3755.

Duerre P, Andreesen JR. 1982. Selenium-dependent growth and glycine fermentation by *Clostridium purinolyticum*. J Gen Microbiol 128:1457–1466.

Enoch HG, Lester RL. 1972. Effects of molybdate, tungstate, and selenium compounds on formate dehydrogenase and other enzymes in *Escherichia coli*. J Bacteriol 110:1032–1040.

Falcone G, Nickerson WJ. 1963. Reduction of selenite by intact yeast cells and cell-free preparations. J Bacteriol 85:754–762.

Fleming RW, Alexander M. 1972. Dimethyl selenide and dimethyl telluride formation by a strain of *Penicillium*. Appl Microbiol 24:424–429.

Frankenberger WT, Karlson U. 1992. Dissipation of soil selenium by microbial volatilization. In: Adriano DC, ed. Biogeochemistry of Trace Metals. Boca Raton, FL: Lewis Publishers, pp 365–381.

Frankenberger WT, Karlson U. 1995. Soil management factors affecting volatilization of selenium from dewatered sediments. Geomicrobiol J 12:265–277.

Gerrard TL, Telford JN, Williams HH. 1974. Detection of selenium deposits in *Escherichia coli* by electron microscopy. J Bacteriol 119:1057–1060.

Guzzo J, Dubow MS. 2000. A novel selenite- and tellurite-inducible gene in *Escherichia coli*. Appl Environ Microbiol 66:4972–4978.

Huber R, Sacher M, Vollmann A, Huber H, Rose D. 2000. Respiration of arsenate and selenate by hyperthermophilic archaea. Syst Appl Microbiol 23:305–314.

Kovalskii VV, Ermakov VV, Letunova SV. 1968. Geochemical ecology of microorganisms in soils with different selenium content. Mikrobiologiya 37:122–139.

Lansche AM. 1965. Tellurium. In: Mineral Facts and Problems. Washington, DC: Bur Mines, Dept of the Interior, pp 935–939.

Lester RL, DeMoss JA. 1971. Effects of molybdate and selenite on formate and nitrate metabolism in *Escherichia coli*. J Bacteriol 105:1006–1014.

Lipman JG, Waksman SA. 1923. The oxidation of selenium by a new group of autotrophic microorganisms. Science 57:60.

Lortie L, Gould WD, Rajan S, McCready RGL, Cheng J-J. 1992. Reduction of selenate and selenite to elemental selenium by a *Pseudomonas stutzeri* isolate. Appl Environ Microbiol 58:4042–4044.

Macy JM, Rech S, Auling G, Dorsch M, Stackebrandt E, Sly L. 1993. *Thauera selenatis* gen. nov. sp. nov., a member of the beta-subclass of Proteobacteria with a novel type of anaerobic respiration. Int J Syst Bacteriol 43:135–142.

Maiers DT, Wichlacz PL, Thompson DL, Bruhn DF. 1988. Selenate reduction by bacteria from a selenium-rich environment. Appl Environ Microbiol 54:2591–2593.

Masscheleyn PH, Patrick WH Jr. 1993. Biogeochemical processes affecting selenium cycling in wetlands. Environ Toxicol Chem 12:2235–2243.

Mertz W. 1981. The essential trace elements. Science 213:1332–1338.
Miller WJ, Neathery MW. 1977. Newly recognized trace mineral elements and their role in animal nutrition. BioScience 27:674–679.
Nagai S. 1965. Differential reduction of tellurite by growing colonies of normal yeasts and respiration deficient mutants. J Bacteriol 90:220–222.
Nelson DC, Casey WH, Sison JD, Mack EE, Ahmad A, Pollack JS. 1996. Selenium uptake by sulfur-accumulating bacteria. Geochim. Cosmochim. Acta 60:3531–3539.
Newman DK, Ahmann D, Morel FMM. 1998. A brief review of microbial arsenate respiration. Geomicrobiol J 15:255–268.
Nickerson, WJ, Falcone G. 1963. Enzymatic reduction of selenite. J Bacteriol 85:763–771.
Oremland RS, Hollibaugh JT, Maest AS, Presser TS, Miller LG, Culbertson CW. 1989. Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: Biogeochemical significance of a novel sulfate-independent respiration. Appl Environ Microbiol 55:2333–2343.
Oremland RS, Steinberg NA, Presser TS, Miller LG. 1991. In situ bacterial selenate reduction in the agricultural drainage system of western Nevada. Appl Environ Microbiol 57:615–617.
Oremland RS, Switzer Blum J, Culbertson CW, Visscher PT, Miller LG, Dowdle P, Strohmaier FE. 1994. Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Appl Environ Microbiol 60:3011–3019.
Oremland RS, Switzer Blum J, Burns Bindi A, Dowdle PR, Herbel M, Stolz JF. 1999. Simultaneous reduction of nitrate and selenate by cell suspensions of selenium-respiring bacteria. Appl Environ Microbiol 65:4385–4392.
Patrick R. 1978. Effects of trace metals in the aquatic ecosystem. Am Sci 66:185–191.
Pinsent J. 1954. The need for selenite and molybdate in the coli-aerogenes group of bacteria. Biochem J 57:10–16.
Rapp G Jr. 1972. Selenium: Element and geochemistry. In: Fairbridge RW, ed. The Encyclopedia of Geochemistry and Environmental Sciences. Encycl Earth Sci Ser; Vol IVA New York: Van Nostrand Reinhold, pp 1079–1080.
Reamer DC, Zoller WH. 1980. Selenium biomethylation products from soil and sewage. Science 208:500–502.
Rech S, Macy JM. 1992. The terminal reductases for selenate and nitrate respiration in *Thauera selenatis* are two distinct enzymes. J Bacteriol 174:7316–7320.
Rosenfeld I, Beath OA. 1964. Selenium, Geobotany, Biochemistry, Toxicity, and Nutrition. New York: Academic Press.
Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafman DG, Hoekstra WG. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588–590.
Roux M, Sarret G, Pignot-Paintrand I, Fontecave M, Coves J. 2001. Mobilization of selenite by *Ralstonia metallidurans* CH34. Appl Environ Microbiol 67:769–773.
Sapozhnikov DI. 1937. The substitution of selenium for sulfur in the photoreduction of carbonic acid by purple sulfur bacteria. Mikrobiologiya 6:643–644.
Sarathchandra SU, Watkinson JH. 1981. Oxidation of elemental selenium to selenite by *Bacillus megaterium*. Science 211:600–601.

Schroeder I, Rech S, Krafft T, Macy JM. 1997. Purification and characterization of the selenate reductase from *Thauera selenatis*. J Biol Chem 272:23765–23768.

Shrift A. 1964. Selenium in nature. Nature (Lond) 201:1304–1305.

Shum AD, Murphy JC. 1972. Effects of selenium compounds on formate metabolism and coincidence of selenium-75 incorporation and formic dehydrogenase activity in cell-free preparations of *Escherichia coli*. J Bacteriol 110:447–449.

Silverman MP, Ehrlich HL. 1964. Microbial formation and degradation of minerals. Adv Appl Microbiol 6:153–206.

Stadtman TC. 1974. Selenium biochemistry. Science 183:915–922.

Steinberg NA, Oremland RS. 1990. Dissimilatory selenate reduction potentials in a diversity of sediment types. Appl Environ Microbiol 56:3550–3557.

Steinberg NA, Blum JS, Hochstein L, Oremland RS. 1992. Nitrate is a preferred electron acceptor for growth of freshwater selenate-respiring bacteria. Appl Environ Microbiol 58:426–428.

Stolz JF, Oremland RS. 1999. Bacterial respiration of arsenic and selenium. FEMS Microbiol Rev 23:615–627.

Stolz JF, Ellis DJ, Switzer Blum J, Ahmann D, Oremland RS, Lovley DR. 1999. *Sulfurospirillum barnesii* sp. nov. and *Sulfurospirillum arsenophilus* sp. nov., new members of the *Sulfurospirillum* clade of the ε-Proteobacteria. Int J Syst Bacteriol 49:1177–1180.

Switzer Blum J, Burns Bindi A, Buzzelli J, Stolz JF, Oremland RS. 1998. *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: Two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30.

Switzer Blum J, Stolz JF, Oren A, Oremland RS. 2001. *Selenihalanaerobacter shriftii* gen. nov., spec. nov., a halophilic anaerobe from Dead Sea sediments that respires selenate. Arch Microbiol 175:208–219.

Thompson-Eagle ET, Frakenberger WT Jr, Karlson U. 1989. Volatilization of selenium by *Alternaria alternata*. Appl Environ Microbiol 55:1406–1413.

Tomei FA, Barton LL, Lemansky CL, Zocco TG. 1992. Reduction of selenate and selenite to elemental selenium by *Wolinella succinogenes*. Can J Microbiol 38:1328–1333.

Torma AE, Habashi F. 1972. Oxidation of copper(II) selenide by *Thiobacillus ferrooxidans*. Can J Microbiol 18:1780–1781.

Trutko SM, Akimenko VK, Suzina NE, Anisimova LA, Shlyapnikov MG, Baskkunov BP, Duda VI, Boronin AM. 2000. Involvement of the respiratory chain of gram-negative bacteria in the reduction of tellurite. Arch Microbiol 173:178–186.

Turner RJ, Hou Y, Weiner JH, Taylor DE. 1992. The arsenical ATPase efflux pump mediates tellurite resistance. J Bacteriol 174:3092–3094.

Van Fleet-Stadler V, Chasteen TG, Pickering IJ, George GN, Prince RC. 2000. Fate of selenate and selenite metabolized by *Rhodobacter sphaeroides*. Appl Environ Microbiol 66:4849–4853.

Völkl P, Huber R, Drobner E, Rachel R, Burggraf S, Trincone A, Stetter KO. 1993. Pyrobaculum aerophilum sp. nov., a novel nitrate-reducing hyperthermophilic archaeum. Appl Environ Microbiol 59:2918–2926.

Woolfolk CA, Whiteley HR. 1962. Reduction of inorganic compounds with molecular hydrogen by *Micrococcus lactilyticus*. I. Stoichiometry with compounds of arsenic, selenium, tellurium, transition and other elements. J Bacteriol 84:647–658.

Yamamoto I, Saiki T, Liu S-M, Ljungdahl LG. 1983. Purification and properties of NADP-dependent formate dehydrogenase from *Clostridium thermoaceticum*, a tungstate-selenium-iron protein. J Biol Chem 258:1826–1832.

Zalokar M. 1953. Reduction of selenite by *Neurospora*. Arch Biochem Biophys 44:330–337.

Zehr JP, Oremland RS. 1987. Reduction of selenate to selenide by sulfate-respiring bacteria: Experiments with cell suspensions and estuarine sediments. Appl Environ Microbiol 53:1365–1369.

21

Geomicrobiology of Fossil Fuels

21.1 INTRODUCTION

Although most organic carbon in the biosphere is continually recycled, a very significant amount has been trapped in special sedimentary formations, where it is inaccessible to mineralization by microbes until it becomes re-exposed to water through natural causes or human intervention. A simultaneous need for access of air depends on the microbes that this carbon encounters and on the electron acceptors other than oxygen that these microbes find available to them. The trapped organic carbon exists in various forms. The degree of its chemically reduced state is related to the length of time it has been trapped and any secondary changes that it has undergone during this time. Some of this carbon has value as a fuel, a source of energy for industrial and other human activity, and is exploited for this purpose. Because of the great age of this material, it is known as *fossil fuel*. The remainder of the trapped carbon is chiefly kerogen and bitumen, some of which can be converted to fuel by human intervention. Fossil fuels include methane gas, natural gas (which is largely methane), petroleum, oil shale, coal, and peat. They are generally considered to have had a microbial origin (Ourisson et al., 1984).

21.2 NATURAL ABUNDANCE OF FOSSIL FUELS

A major portion of the carbon at the Earth's surface is in the form of carbonate (Fig. 21.1). It represents a major sink for carbon. The other sink is the trapped

organic carbon that is not directly accessible for microbial mineralization. The carbonate carbon is not an absolute sink unless it is deeply buried because it is in a steady-state relationship with dissolved carbonate/bicarbonate and atmospheric CO_2, which in turn are in a steady-state relationship with organic carbon in living

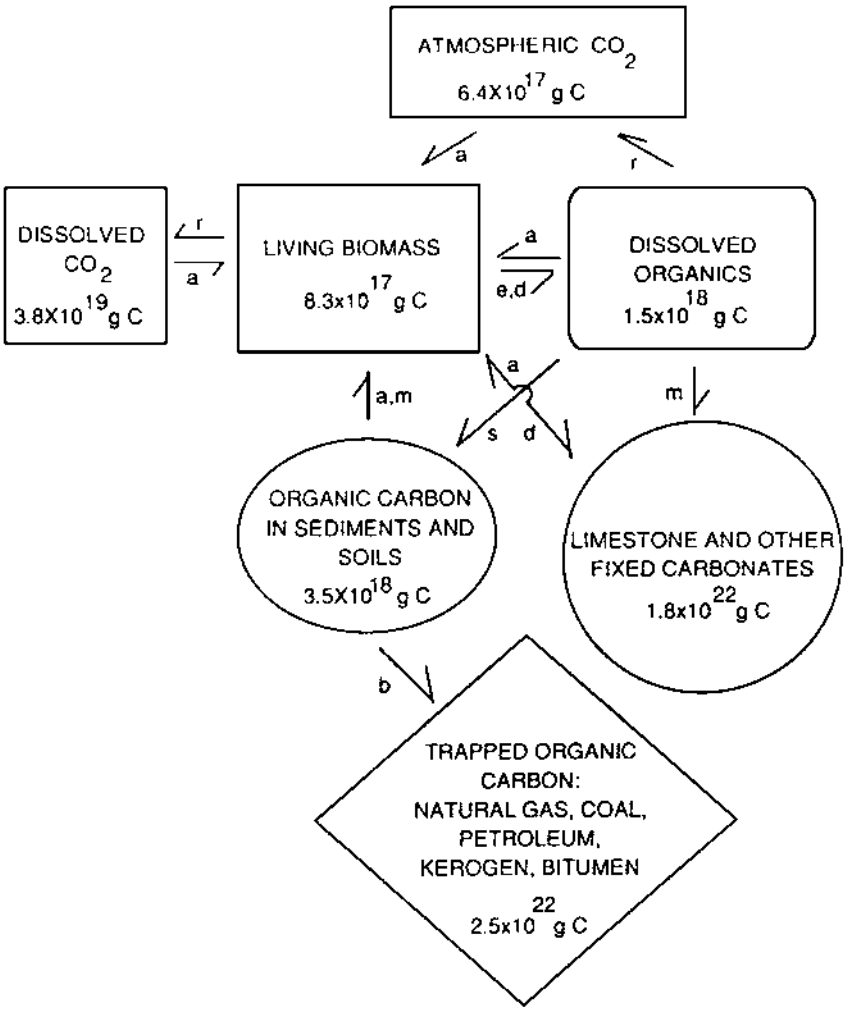


FIG. 21.1 Microbial and physical processes contributing to carbon transfer among different compartments in the biosphere. a, Microbial assimilation; b, burial; d, decomposition; e, excretion; m, microbial mineralization; r, respiration; s, sedimentation. Quantitative estimates from Fenchel and Blackburn (1979) and Bowen (1979).

and dead biomass. The passage of carbon from one compartment into another is under biological control (Fig. 21.1) (Fenchel and Blackburn, 1979).

21.3 METHANE

Methane at atmospheric pressure and ambient temperature is a colorless, odorless, and flammable gas. Because its autoignition temperature is 650°C, it does not catch fire spontaneously. It is sparingly soluble in water (3.5 mL per 100 mL of water) but readily soluble in organic solvents, including liquid hydrocarbons. Biogenic accumulations may occur in nature when methane is formed in consolidated sediment from which it cannot readily escape. Its formation comes about when organic matter in the sediment is undergoing anaerobic microbial mineralization in the absence of significant quantities of alternative terminal electron acceptors such as nitrate, Fe(III), Mn(IV), or sulfate. If gas pressure due to methane builds up sufficiently in anaerobic lake or coastal sediment, it may be seen to escape in the form of large gas bubbles that break at the water surface to release their methane into the atmosphere (Martens, 1976; Zeikus, 1977). In marshes, escaping methane may be ignited (by biogenic phosphene?) to burn continually as so-called will-o'-the-wisps.

Many of the methane accumulations on Earth are of biogenic origin. Methane may occur in association with peat, coal, and oil deposits or independently of them. That which occurs in association with coal and oil was probably microbially generated in the early stages of their formation, although some may have been formed abiotically in later diagenetic phases. Methane associated with coal deposits can be the cause of serious mine explosions when accidentally ignited. It is called *coal damp* by coal miners.

Biogenic methane formation is a unique biochemical process that appears to have arisen very early in the evolution of life. Indeed, the methanogenesis that results from the reduction of CO_2 and H_2 may represent the first or one of the first autotrophic processes on Earth (see Chap. 3).

Methanogens

All methane-forming bacteria, i.e., **methanogens**, are members of the domain Archaea. As a group, they are very diverse phylogenetically (Jones et al., 1987; Boone et al., 1993). They also show great diversity morphologically, existing as rods, spirilla, cocci, and sarcinae (Fig. 21.2). The feature that they share in common is that of being strict anaerobes that form methane in their respiration. The large majority of them are obligate or facultative autotrophs. They can get their energy from the reduction of carbon dioxide with hydrogen or its equivalent

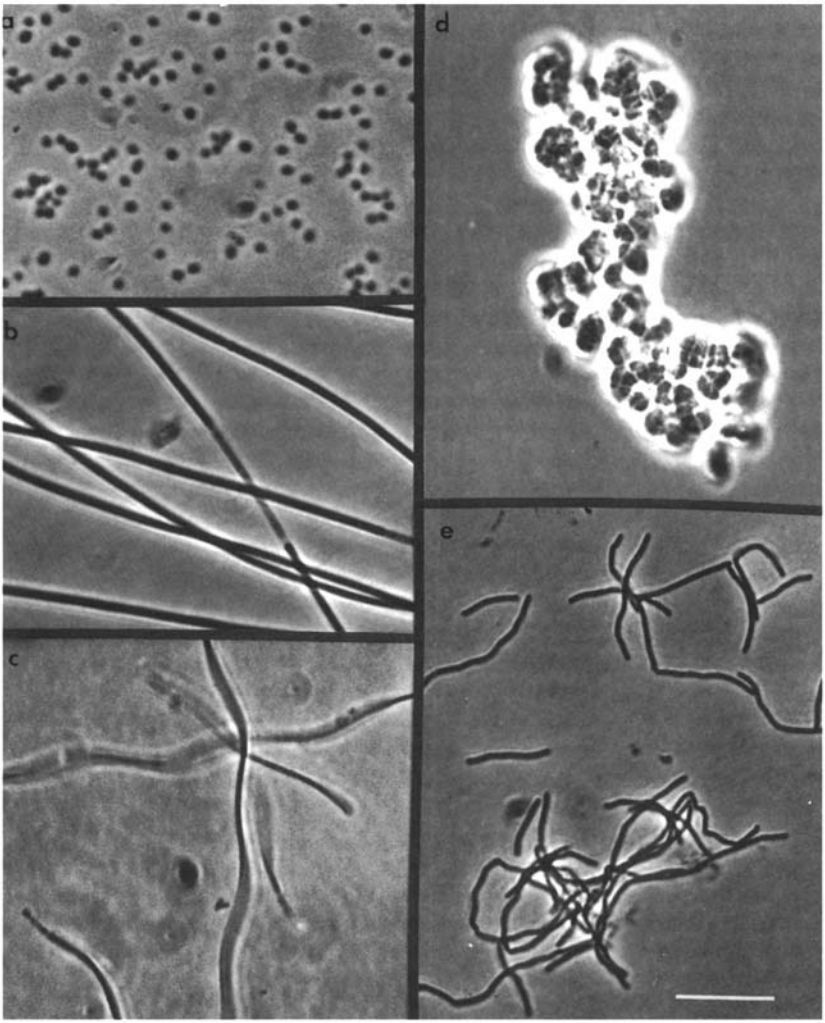


FIG. 21.2 Morphologies of different methanogens. a, *Methanocullens bourgensis* (formerly *Methanogenium bourgense*); b, *Methanosaeta* (formerly *Methanothrix) concilii*: c, *Methanobacterium hungatei* OGC 16; d, *Methanosarcina barkeri* OGC 35; e, *Methanobacterium formicicum* OGC 55. Scale mark represents 10 μm and applies to all panels. (Courtesy of David R. Boone and Ron Willis.)

(formate or CO), whereas they get their carbon exclusively by assimilating carbon dioxide. However, some, like *Methanosarcina* (formerly *Methanothrix*) *soehngii* (Zehnder et al., 1980; Huser et al., 1982) and *Methanosaeta* (formerly *Methanothrix*) *concilii* (Patel, 1984), are heterotrophs requiring acetate as energy source and carbon source and for this reason are known as *acetotrophic* or *aceticlastic* methanogens. At least one methanogen can use S^0 in addition to CO_2 as terminal electron acceptor (Stetter and Gaag, 1983). As a group, most methanogens are nutritionally restricted to the following energy sources: H_2, CO, HCOOH, methanol, methylamines, and acetate (Atlas, 1997; Brock and Madigan, 1988). But exceptions exist. Widdel reported that a freshwater strain of *Methanospirillum* and a strain of *Methanogenium* were each able to grow on 2-propanol and 2-butanol as well as on H_2 and formate. Zellner et al. (1989) found that *Methanobacterium palustre* was able to grow on 2-propanol as energy source as well as H_2 and formate. It was able to oxidize but not grow on 2-butanol. In 1990, Zellner et al. reported that *Methanogenium liminatans* can use 2-propanol, 2-butanol, and cyclopentanol as energy sources in addition to H_2 and formate. Finster et al. (1992) found that strain MTP4 can use methanediol and dimethylsulfide as well as methylamines, methanol, and acetate as energy sources. Yang et al. (1992) found that *Methanococcus voltae*, *M. maripaludis*, and *M. vannielii* can each use pyruvate as energy source in the absence of H_2.

In order for methanogens to be able to draw on the wide range of oxidizable carbon compounds that may be available in their environment but that they cannot metabolize directly, they associate with heterotrophic fermenters and/or anaerobic respirers that do not completely mineralize their organic energy sources (see, e.g., Jain and Zeikus, 1989; Sharak Genthner et al., 1989; Grbic-Galic, 1990). To optimize access to the microbially generated energy sources that they need, some methanogens form intimate consortia (*syntrophic associations*) with other anaerobic bacteria that can furnish them with these nutrients (metabolites), which they form among their metabolic end products (see, e.g., Bochem et al., 1982; MacLeod et al., 1990; McInerney et al., 1979; Winter and Wolfe, 1979, 1980; Zinder and Koch, 1984; Wolin and Miller, 1987). Frequently the metabolites that are the basis for these syntrophic associations are not readily detectable when all the members of the consortium are growing together in a mixed culture. This is because they are consumed as quickly as they are formed. When hydrogen is the metabolite, the process is called interspecies hydrogen transfer (Wolin and Miller, 1987).

Among the most widely recognized genera of methanogens are *Methanobacterium*, *Methanothermobacter*, *Methanobrevibacterium*, *Methanococcus*, *Methanomicrobium*, *Methanogenium*, *Methanospirillum*, *Methanosarcina*, *Methanoculleus*, and *Methanosaeta* (see Brock and Madigan, 1988; Bhatnagar et al., 1991; Boone et al., 1993; Atlas, 1997). Methanogens may be mesophilic or thermophilic. They are found in diverse anaerobic habitats (Zinder, 1993),

including marine environments such as salt marsh sediments (Oremland et al., 1982; Jones et al., 1983b), coastal sediments (Gorlatov et al., 1986; Sansone and Martens, 1981), anoxic basins (Romesser et al., 1979), geothermally heated seafloor (Huber et al., 1982), hydrothermal vent effluent on the East Pacific Rise (Jones et al., 1983a), sediment effluent channel of the Crystal River Nuclear Power Plant (Florida) (Rivard and Smith, 1982), lakes (Deuser et al., 1973; Jones et al., 1982; Giani et al., 1984), soils (Jakobsen et al., 1981), desert environments (Worakit et al., 1986), solfataric fields (Wildgruber et al., 1982; Zabel et al., 1984), oil deposits (Nazina and Rozanova, 1980; Rubinshtein and Oborin, 1986; Stetter et al., 1993), the digestive tract of insects and higher animals, especially ruminants and other herbivores (Breznak, 1982; Brock and Madigan, 1988; Wolin, 1981; Zimmerman et al., 1982; Atlas, 1997), and as endosymbionts (van Bruggen et al., 1984; Fenchel and Finlay, 1992). Thus, despite their obligately anaerobic nature, methanogens are fairly ubiquitous.

Methanogens play an important but not exclusive role in anaerobic mineralization of organic carbon compounds in soil and aquatic environments, especially freshwater sediments (Wolin and Miller, 1987). In marine sediments, where methanogens have to share hydrogen and/or acetate as sources of energy with sulfate-reducing bacteria, they tend to be outcompeted by the sulfate reducers because of the latter's higher affinity for hydrogen and acetate (Abrams and Nedwell, 1978; Kristjanssen et al., 1982; Schönheit et al., 1982; Robinson and Tiedje, 1984). Thus in many estuarine or coastal anaerobic muds, sulfate-reducing activity and methanogenesis occur usually in spatially separated zones in the sediment profile, with the zone exhibiting sulfate-reducing activity overlying the zone exhibiting methanogenesis (e.g., Martens and Berner, 1974; Sansone and Martens, 1981). Some indirect evidence has been interpreted to indicate that some sulfate-reducing bacteria can also use methane as electron donor (see section below on methane oxidation).

Under two special circumstances, methanogenesis and sulfate reduction can be compatible in an anaerobic marine environment. One circumstance is the existence of an excess supply of a shared energy source (H_2 or acetate) (Oremland and Taylor, 1978). The other circumstance is one where sulfate reducers and methanogens use different energy sources, namely products of decaying plant material and methanol or trimethylamine, respectively (Oremland et al., 1982). In anaerobic freshwater sediments and soils where sulfate, nitrate, ferric(III) oxide, and manganese(IV) oxide concentrations are very low, methanogenesis is usually the dominant mechanism of organic carbon mineralization. Yet even here, certain sulfate-reducing bacteria may grow in the same niche as methanogens. Indeed, they may form a consortium with them. In the absence of sulfate, these sulfate reducers ferment suitable organic carbon with the production of H_2 as one of the results of their energy metabolism, which the methanogens then use in their energy metabolism to form methane (e.g., Bryant et al., 1977).

Methanogenesis and Carbon Assimilation by Methanogens

Methanogenesis

Methane formation represents a form of anaerobic respiration in which CO_2 is usually the terminal electron acceptor. In the simplest case where hydrogen is the energy source (electron donor), it reduces CO_2 to methane according to the overall reaction

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{21.1}$$

This reaction is exothermic ($\Delta G^\circ = -33$ kcal or $-137.9\ kJ$) and yields energy that can be used by the organism to do metabolic work. Reactions utilizing formic or acetic acid, methanol, or methylamines as electron acceptor instead of CO_2 follow essentially the same principle:

$$HCOOH + 3H_2 \rightarrow CH_4 + 2H_2O \quad (\Delta G^\circ = -42\,\text{kcal or } -175.6\,\text{kJ}) \tag{21.2}$$

$$CH_3COOH + 4H_2 \rightarrow 2CH_4 + 2H_2O \quad (\Delta G^\circ = -49\,\text{kcal or } -204.8\,\text{kJ}) \tag{21.3}$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \quad (\Delta G^\circ = -26.9\,\text{kcal or } -112.4\,\text{kJ}) \tag{21.4}$$

$$CH_3NH_2 + H_2 \rightarrow CH_4 + NH_3 \quad (\Delta G^\circ = -9\,\text{kcal or } -37.6\,\text{kJ}) \tag{21.5}$$

Here each of the carbons is reduced to methane by hydrogen.

In a few instances, secondary alcohols were found to serve as electron donors, with CO_2 as the terminal electron acceptor. The CO_2 was therefore the source of the methane formed (Widdel, 1986; Zellner et al., 1989). In these reactions, the alcohols were replacing H_2 as the reductant of CO_2.

Some methanogens can also form methane from carbon monoxide, formic acid, methanol, acetate, or methylamines without H_2 as electron donor. In these instances they perform disproportionations (fermentations) in which a portion of the substrate molecules act as electron donors (energy source) and the rest as

electron acceptors. For instance (Brock and Madigan, 1988; Atlas, 1997; Mah et al., 1978; Smith and Mah, 1978; Zeikus, 1977),

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O \quad (\Delta G^\circ = -35\,\text{kcal or } -146.3\,\text{kJ}) \tag{21.6}$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (\Delta G^\circ = -76\,\text{kcal or } -317.7\,\text{kJ}) \tag{21.7}$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (\Delta G^\circ = -9\,\text{kcal or } -37.6\,\text{kJ}) \tag{21.8}$$

$$4CH_3NH_2 + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_3 \quad (\Delta G^\circ = -75\,\text{kcal or } -313.5\,\text{kJ}) \tag{21.9}$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \quad (\Delta G^\circ = -44.5\,\text{kcal or } -186\,\text{kJ}) \tag{21.10}$$

Some methanogens can even form methane from pyruvate by disproportionation (Yang et al., 1992). Resting cells of *Methanococcus* spp. grown in pyruvate-containing medium in an N_2 atmosphere were shown to transform pyruvate to acetate, methane, and CO_2 according to the following stoichiometry (Yang et al., 1992):

$$4CH_3COCOOH + 2H_2O \rightarrow 4CH_3COOH + 3CO_2 + CH_4 \quad (\Delta G^\circ = -74.9 \text{ or } 313.1\,\text{kJ}) \tag{21.11}$$

This stoichiometry is attained if the organism oxidatively decarboxylates pyruvate:

$$4CH_3COCOOH + 4H_2O \rightarrow 4CH_3COOH + 4CO_2 + 8(H) \tag{21.11a}$$

and uses the reducing power [8(H)] to reduce one-fourth of the CO_2 to CH_4:

$$CO_2 + 8(H) \rightarrow CH_4 + 2H_2O \tag{21.11b}$$

Bock et al. (1994) found that a spontaneous mutant of *Methanosarcina barkeri* could grow by fermenting pyruvate to methane and CO_2 with the following stoichiometry:

$$CH_3COCOOH + 0.5H_2O \rightarrow 1.25CH_4 + 1.75CO_2 \tag{21.12}$$

To achieve this stoichiometry, the authors proposed the following mechanism based on known enzyme reactions in methanogens. Pyruvate is oxidatively decarboxylated to acetyl $\sim$ SCoA and CO_2:

$$CH_3COCOOH + CoASH \rightarrow CH_3CO \sim SCoA + CO_2 + 2(H) \tag{21.12a}$$

The available reducing power [2(H)] from this reaction is then used to reduce one-fourth of the CO_2 formed to methane:

$$0.25CO_2 + 2(H) \rightarrow 0.25CH_4 + 0.5H_2O \quad (21.12b)$$

and the acetyl~SCoA is decarboxylated to methane and CO_2:

$$CH_3CO \sim SCoA + H_2O \rightarrow CH_4 + CO_2 + CoASH \quad (21.12c)$$

The standard free energy yield at pH 7 ($\Delta G^{\circ\prime}$) was calculated to be $-22.9\,kcal\,mol^{-1}$ or $-96\,kJ\,mol^{-1}$ of methane produced (Bock et al., 1994).

Although reactions (21.1)–(21.11) look very disparate, they share a common metabolic pathway (Fig. 21.3). The reason methanogens differ with respect to the methane-forming reactions they can perform is that they do not all possess the same key enzymes that permit entry of particular methanogenic substrates into the common pathway (Vogels and Visser, 1983; Zeikus et al., 1985; Stanier et al., 1986; Brock and Madigan, 1988; Atlas, 1997). The pathway involves stepwise reduction of carbon from the +4 to the −4 oxidation state via bound formyl, methylene, and methyl carbon. The operation of the methane-forming pathway requires some unique coenzyme and carrier molecules (Table 21.1). Coenzyme M (2-mercaptoethylsulfonate) is unique to methanogens and may be used to identify them as methane formers. The large majority of methanogens synthesize this molecule de novo.

New evidence suggests that reaction (21.1) can occur abiotically in the presence of a nickel-iron alloy under hydrothermal conditions (e.g., 200–400°C, 50 MPa), conditions met in parts of the oceanic crust, for instance (Horita and Berndt, 1999).

TABLE 21.1 Unusual Coenzymes in Methanogens

Coenzyme[a]	Function
Methanofuran	CO_2 reduction factor in first step of methanogenesis
Methanopterin (coenzyme F_{342})	Formyl and methene carrier in methanogenesis
Coenzyme M (2-mercaptoethane sulfonate)	Methyl carrier in methanogenesis
Coenzyme F_{430}	Hydrogen carrier for reduction of methyl coenzyme M
Coenzyme F_{420} (nickel-containing tetrapyrrole)	Mediates electron transfer between hydrogenase or formate and NADP; reductive carboxylation of acetyl~CoA and succinyl~CoA

[a] For structures of these coenzymes, see Brock and Madigan (1988) and Blaut et al. (1992).

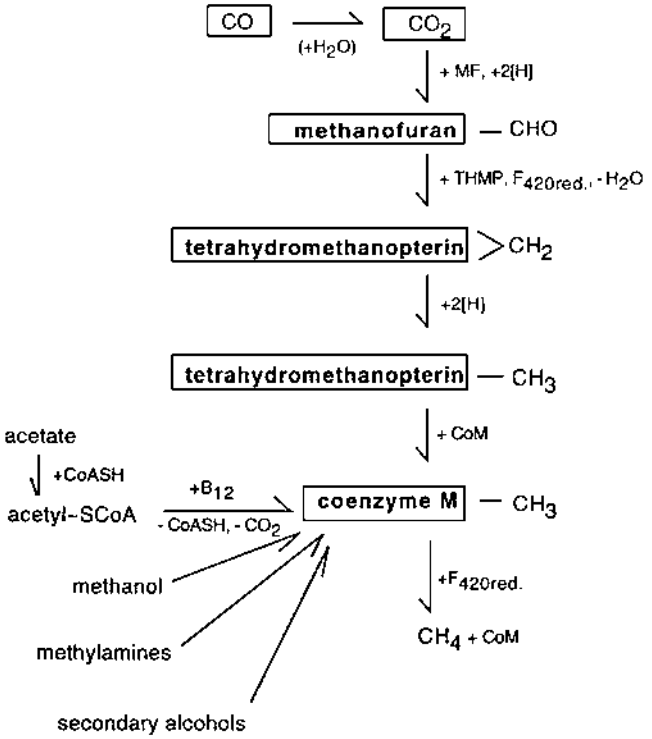


FIG. 21.3 Pathways of methanogenesis from CO, CO_2, acetate, methanol, and methylamines.

Bioenergetics of Methanogenesis

As an anaerobic respiratory process, methane formation is performed to yield useful energy to the cell. Evidence to date indicates that ATP is generated by chemiosmotic energy-coupling metabolism (e.g., Mountford, 1978; Doddema et al., 1978, 1979; Blaut and Gottschalk, 1984; Sprott et al., 1985; Gottschalk and Blaut, 1990; Blaut et al., 1990, 1992; Müller et al., 1993; Atlas, 1997). The chemiosmotic coupling mechanism may involve protons or sodium ions. Membrane-associated electron transport constituents required in chemiosmotic energy conservation involving proton coupling in *Methanosarcina* strain Gö 1 include reduced factor F_{420} dehydrogenase, an unknown electron carrier, cytochrome b, and heterodisulfide reductase (see Blaut et al., 1992). The heterodisulfide consists of coenzyme M covalently linked to 7-mercaptoheptanoylthreonine by a disulfide bond (Blaut et al., 1992). A proton-translocat-

ing ATPase associated with the membrane catalyzes ATP synthesis in this organism.

An example of a methanogen that employs sodium ion coupling is *Methanococcus voltae* (Dybas and Konisky, 1992; Chen and Konisky, 1993). It appears to employ Na^+-translocating ATPase that is insensitive to proton translocation inhibitors. A scheme for pumping sodium ions from the cytoplasm to the periplasm that depends on a membrane-bound methyl transferase was proposed by Blaut et al. (1992).

Carbon Fixation by Methanogens

When methanogens grow autotrophically, their carbon source is CO_2. The mechanism by which they assimilate CO_2 is different from that of most autotrophs (Simpson and Whitman, 1993). Most autotrophs in the domain Bacteria use the pentose diphosphate pathway (Calvin–Benson cycle). Among the exceptions are the green sulfur bacteria, which use a reverse tricarboxylic acid cycle, and the methane-oxidizing bacteria, which use either the hexulose monophosphate or the serine pathway (see next section). Methanogens, like homoacetogens (see Chap. 6) and some sulfate-reducing bacteria (see Chap. 18), assimilate carbon by reducing one of two molecules of CO_2 to methyl carbon and the second to a formyl carbon. They then couple the formyl carbon to the methyl carbon to form acetyl~SCoA (see Fig. 6.8 in Chap. 6). To form the important metabolic intermediate pyruvate, they next carboxylate the acetyl~SCoA reductively. All other cellular constituents are then synthesized from pyruvate (Simpson and Whitman, 1993).

Microbial Methane Oxidation

Methanotrophs

Methane can be used as a primary energy source by a number of aerobic bacteria. Some of these are obligate **methanotrophs**; others are facultative (Higgins et al., 1981). Methane is also oxidized by some yeasts (Higgins et al., 1981).

Strong indirect evidence has been amassed that methane can also be oxidized anaerobically in some marine environments. Sulfate-reducing bacteria have been implicated in this oxidation on the assumption that some of them can use methane as electron donor for the reduction of sulfate (e.g., Oremland and Taylor, 1978; Panganiban et al., 1979; Reeburgh, 1980; Martens and Klump, 1984; Iverson and Jørgensen, 1985; Gal'chenko et al., 1986; Henrichs and Reeburgh, 1987; Ward et al., 1987). Only one sulfate-reducing culture with such an ability has been isolated so far (Panganiban and Hanson, 1976; Panganiban et al., 1979). Thomsen et al. (2001), who studied anaerobic sediments from Aarhus Bay, have suggested that an archaeal sulfate reducer with methane-

oxidizing capacity could have been active in their samples but were unable rule out other possibilities. Hoehler et al. (1994, 1998), Hansen et al. (1998), Niewöhner et al. (1998), and Pancost et al. (2000) suggested that anaerobic methane oxidation may involve a consortium of methanogens and sulfate reducers. This was also an alternative interpretation by Thomsen et al. (2001) of their data. A recent rRNA gene and lipid analysis of anoxic methane seep sediments from the California continental margin suggested that members of Methanosarcinales and Desulfosarcinales may be active in such consortia (Orphan et al., 2001). In this system, methanogens would oxidize methane anaerobically by reversing the methanogenic reaction

$$CH_4 + 2H_2O \rightarrow CO_2 + 4H_2 \tag{21.13}$$

Sulfate reducers would immediately consume the H_2 formed in reaction (21.13);

$$HSO_4^- + 4H_2 \rightarrow HS^- + 4H_2O \tag{21.14}$$

It is difficult to visualize reaction (21.13) as a simple reversal of the energy-yielding reaction (21.1) that enables hydrogen-oxidizing methanogens to grow. Reaction (21.13) should consume energy if it uses the same enzymes that are used in formation of methane from CO_2 and H_2. Zehnder and Brock (1979) did observe such a reaction with a methanogenic culture under laboratory conditions, but it was very weak. Reaction (21.13) as written may merely represent an overall reaction of a unique enzymatic pathway in those methanogens that have an ability to oxidize methane anaerobically as well as to form it. Although the combination of reactions (21.13) and (21.14) is inconsistent with an incompatibility of methanogenesis and sulfate reduction (see previous section on methanogens), it would explain the absence of a very low concentration of methane in sulfate-reducing zones overlying methanogenic zones.

Except for the sulfate reducers and methanogens, all known methanotrophs are aerobes. Examples of obligate methanotrophs are *Methylomonas*, *Methylococcus*, *Methylobacter*, *Methylosinus*, and *Methylocystis* (Fig. 21.4). All are gram-negative and feature intracytoplasmic membranes. On the basis of the organization of these membranes, each obligate methanotroph can be assigned to either one or the other of two groups (Davies and Whittenbury, 1970). *Type I* have stacked membranes, whereas *Type II* have paired membranes concentric with the plasma membrane and forming vesicle-like or tubular structures (Fig. 21.5). Facultative methanotrophs feature internal membranes with an appearance like those of the Type II obligate methanotrophs. All methanotrophs can also use methanol as a primary energy source, but not all methanol oxidizers can grow with methane as a primary energy source. Methanol oxidizers that cannot oxidize methane are called **methylotrophs**.

Methanotrophs are important for the carbon cycle in returning the carbon of methane, which is always generated anaerobically, to the reservoir of CO_2 (e.g., Vogels, 1979). Obligate methanotrophs are found at aerobic/anaerobic interfaces

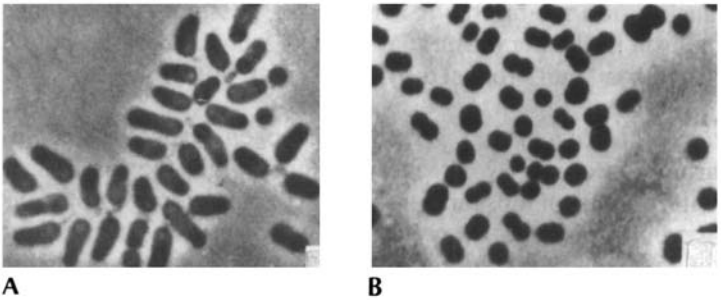


FIG. 21.4 Methane-oxidizing bacteria (methanotrophs) (×19,000). (A) *Methylosinus trichosporium* in rosette arrangement. Organisms are anchored by visible hold fast material. (B) *Methylococcus capsulatus*. (From Whittenbury R, Phillips KC, Wilkinson JF. Enrichment and isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 61:205–218, 1970; with permission.)

in soil and aquatic environments that are crossed by methane (e.g., Alexander, 1977; Reeburgh, 1976; Ward and Brock, 1978; Sieburth et al., 1987) and also in coal and petroleum deposits (Ivanov et al., 1978; Kuznetsov et al., 1963). Some methanotrophs are also important intracellular endosymbionts in marine seep mussels and other benthic invertebrates. Cavanaugh et al. (1987) found evidence of the presence of such symbiotic methanotrophs in the epithelial cells of the gills of some mussels from reducing sediments at hypersaline seeps at abyssal depths

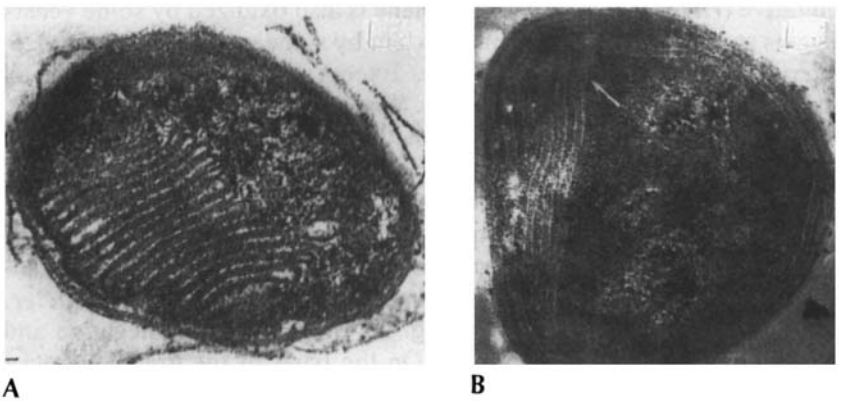


FIG. 21.5 Fine structure of methane-oxidizing bacteria (×80,000). (A) Section of *Methylococcus* (subgroup minimus) showing Type I membrane system. (B) Peripheral arrangement of membranes in *Methylosinus* (subgroup sporium) characteristic of Type II membrane systems. (From Davies SL, Whittenbury R. Fine structure of methane and other hydrocarbon-utilizing bacteria. J Gen Microbiol 61:227–232, 1970; with permission.)

in the Gulf of Mexico at the Florida Escarpment. MacDonald et al. (1990) made similar observations in mussels that occurred in a large bed surrounding a pool of hypersaline water rich in methane at a depth of 650 m on the continental slope south of Louisiana. Transmission electron microscopic examination by Cavanaugh et al. (1987) showed that the symbionts feature typical intracytoplasmic membranes of Type I methanotrophs. They possess the key enzymes associated with methane oxidation in that group (see below). The basis for the symbiosis between the invertebrate host and the methanogen is the sharing of fixed carbon derived from the methane taken up by the host and metabolized by the methanotroph (Childress et al., 1986). This activity is similar to that of the symbiotic H_2S-oxidizing bacteria in some invertebrates of hydrothermal vent communities, which share with their host the carbon they fix from CO_2 taken up by the host (see Chap. 19). Whether the source of the methane issuing from Gulf of Mexico seeps is biogenic or abiogenic is unclear at this time. The invertebrate fauna in the vicinity of hydrocarbon seeps on the Louisiana slope in the Gulf of Mexico features intracellular methanotrophic bacterial endosymbionts only in mussels. Vestimentiferan worms and three clam species in these locations feature autotrophic bacterial sulfur-metabolizing symbionts (Brooks et al., 1987). Some animals at the Oregon subduction zone have also formed associations with methanotrophs that enable them to feed on methane (Kulm et al., 1986).

Biochemistry of Methane Oxidation

Obligate methanotrophs can use methane, methanol, and methylamines as energy sources by oxidizing them to CO_2, H_2O, and NH_3, respectively. When the methane is the energy source, the following steps are involved in its oxidation:

$$CH_4 \xrightarrow{0.5O_2} CH_3OH \xrightarrow{0.5O_2} HCHO \xrightarrow{0.5O_2} HCOOH \xrightarrow{0.5O_2} CO_2 + H_2O \tag{21.15}$$

The first step in this reaction sequence is catalyzed by a monooxygenase that causes the direct introduction of an atom of molecular oxygen into the methane molecule (Anthony, 1986). This step is generally considered not to yield useful energy to the cell. A report by Sokolov (1986), however, suggests the contrary, at least with *Methylomonas alba* BG8 and *Methylosinus trichosporium* OB3b. Because monooxygenase requires pyridine nucleotide ($NADH + H^+$) in its catalytic process to provide electrons for reduction of one of the two oxygen atoms in O_2 to H_2O (the other oxygen atom is introduced into methane to form methanol), a proton motive force is generated in the electron transfer from the reduced pyridine nucleotide, which the cell may be able to couple to ATP synthesis. The enzyme that catalyzes methanol oxidation is methanol dehydrogenase, which in *Methylococcus thermophilus*, as in other methanotrophs, does not use pyridine nucleotide as cofactor (Anthony, 1986; Sokolov et al., 1981).

Instead, the enzyme contains pyrroloquinone (+90 mV) as its prosthetic group, which feeds electrons from methanol into the electron transport chain. The formaldehyde resulting from methanol oxidation is oxidized to formate [reaction (21.15)] (see Roitsch and Stolp, 1985). The oxidation of formaldehyde to formate may involve a pyridine nucleotide-linked dehydrogenase or a pyrroloquinone-linked dehydrogenase (Stanier et al., 1986). Whatever the mechanism of formaldehyde oxidation, the reducing power is fed into the electron transport system for energy generation. The formate dehydrogenase is a pyridine nucleotide-coupled enzyme that oxidizes formate to CO_2 and H_2O and feeds electrons into the electron transport system to generate energy. ATP synthesis in methanotrophs appears to be mainly or entirely by chemiosmosis (Anthony, 1986).

It should be noted that the methane monooxygenase of methanotrophs is not a very specific enzyme. It can also catalyze NH_3 oxidation (O'Neill and Wilkinson, 1977). In this instance, the monooxygenase hydroxylates ammonia to NH_2OH. Ammonia-oxidizing autotrophic bacteria can similarly oxidize methane to methanol (Jones and Morita, 1983). However, just as ammonia oxidizers cannot grow on methane as energy source, methanotrophs cannot grow on ammonia as energy source. This is because they lack the enzyme sequences for methanol or hydroxylamine oxidation, respectively.

Carbon Assimilation by Methanotrophs

All autotrophically grown methanotrophs of Types I and II assimilate some carbon (up to 30%) in the form of CO_2 (Romanovskaya et al., 1980). The enzyme involved in the fixation appears to be phosphoenolpyruvate carboxylase (PEP carboxylase). The mechanism of fixation of the remaining carbon depends on the methanotroph type. Both types assimilate it at the formaldehyde oxidation state. Type I fixes this carbon via an assimilatory, cyclic ribulose monophosphate pathway (Fig. 21.6A) whereas Type II fixes it via a cyclical serine pathway (Fig. 21.6B). In the ribulose monophosphate pathway, 3-phosphoglyceraldehyde is the key intermediate in carbon assimilation, whereas in the serine pathway it is acetyl~SCoA (Brock and Madigan, 1988; Stanier et al., 1986; Gottschalk, 1986; Atlas, 1997). Reducing power for assimilation derives from methane dissimilation and may require reverse electron transport to generate needed $NADPH + H^+$. Methylotrophs generally use the serine pathway for carbon assimilation from C_1 compounds.

Obligate methanotrophs stand somewhere between typical autotrophs and heterotrophs in their carbon assimilating mechanism (Quayle and Ferenci, 1978).

The Position of Methane in the Carbon Cycle

In order for a sufficient pool of biologically available carbon to be maintained in the biosphere, carbon has to be continually recycled from organic to inorganic

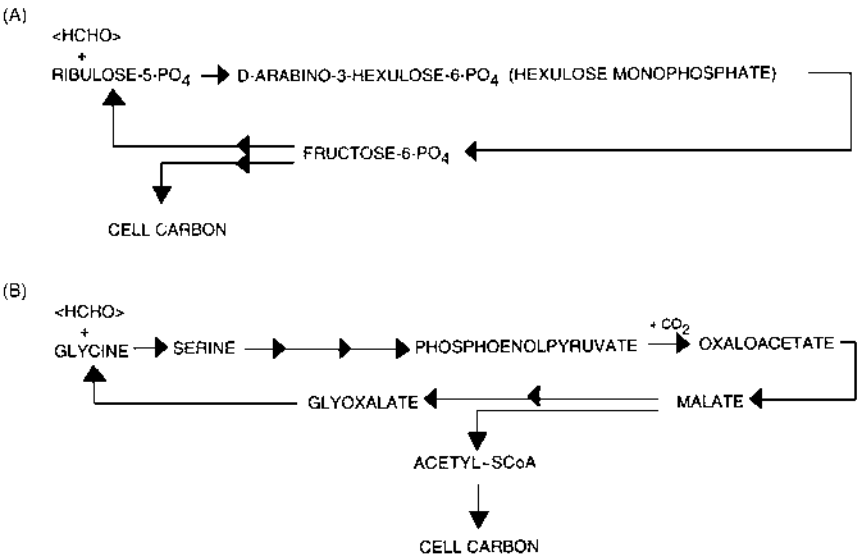


FIG. 21.6 Alternative pathways of formaldehyde carbon assimilation in methanotrophs. The hexulose monophosphate (HuMP) pathway (A) is used by Type I methanotrophs, and the serine pathway (B) is used by Type II methanotrophs.

carbon. This is accomplished both aerobically and anaerobically. Quantitatively, the aerobic process makes the greater contribution, but the anaerobic process is not negligible; indeed, anaerobic mineralization in sediment has been estimated to equal approximately the rate of burial of organic carbon (Henrichs and Reeburgh, 1987). Anaerobic mineralization involves fermentation processes coupled with anaerobic forms of respiration, including nitrate reduction (nitrate respiration, denitrification, nitrate ammonification) (e.g., Sørensen, 1987), iron and manganese respiration (Lovley and Phillips, 1988; Myers and Nealson, 1988; Ehrlich, 1993; Lovley, 1993), iron reduction (Lovley, 1987, 1993), sulfate respiration (Skyring, 1987), and methanogenesis (Wolin and Miller, 1987; Young and Frazer, 1987). Each of these forms of respiration is dominant where the respective electron acceptor is dominant and other environmental conditions are optimal. In some instances, more than one form of anaerobic respiration may occur simultaneously in the same general environment, provided there is no competition for the same electron donor or growth-limiting substance (see Ehrlich, 1993).

In anoxic soils (paddy soils) or anoxic freshwater or marine sediments where extensive methanogenesis occurs, a small portion of the methane is oxidized to CO_2 without the benefit of oxygen. However, a larger portion of the methane that is not trapped escapes into an oxidizing environment and is

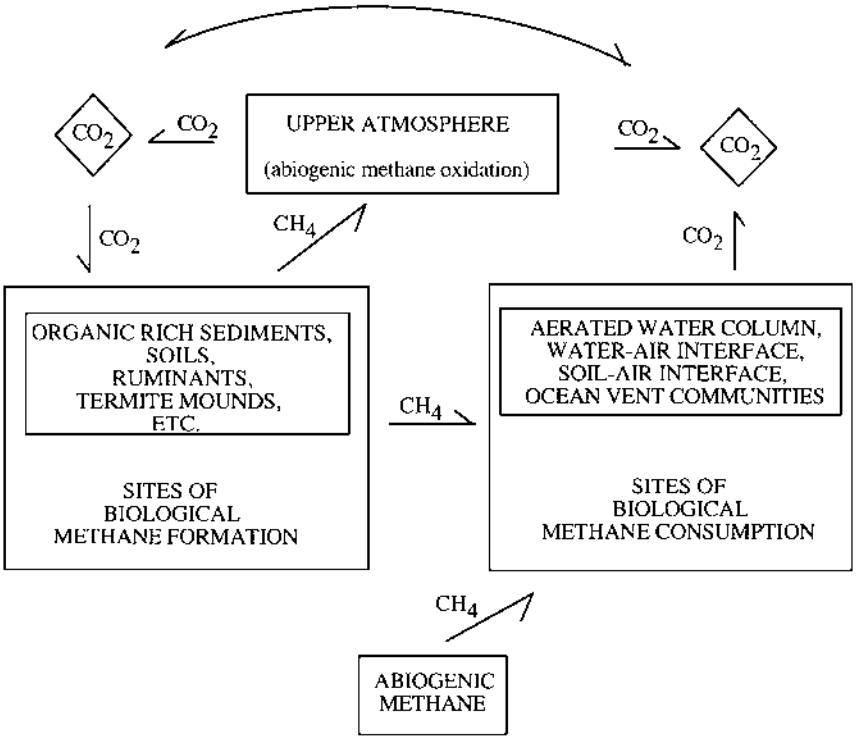


FIG. 21.7 The methane cycle, emphasizing methanogenesis and methanotrophy.

extensively oxidized to CO_2 by aerobic methanotrophs (Higgins et al., 1981). A small amount of methane may be used as energy and carbon source by special marine invertebrates. Any methane that is not bio-oxidized or otherwise combusted or trapped in natural sedimentary reservoirs escapes this biological attack and enters the atmosphere, where it may be chemically oxidized in the troposphere (Vogels, 1979). The various paths for methanogenesis and methane oxidation are summarized in Figure 21.7.

21.4 PEAT

Nature of Peat

Although peat and coal are two different substances, their modes of origin have included common initial steps. Indeed, the formation of peat may have been an intermediate step in the formation of coal. Peat is a form of organic soil or

histosol. It is mostly derived from plant remains that have accumulated in marshes and bogs (Fig. 21.8). According to Francis (1954), these remains have come from (1) sphagnum, grasses, and heather (high moor peat); (2) reeds, grasses, sedges, shrubs, and bushes (low moor peat); (3) trees, branches, and debris of large forests in low-lying wet ground (forest peat); or (4) plant debris accumulated in swamps (sedimentary or lake peat). In all these instances, plant growth outstripped the decay of the plant remains, ensuring a continual supply of new raw material for peat formation.

Role of Microbes in Peat Formation

Initially, the plant remains may have undergone attack by some of their own enzymes but soon were attacked by fungi, which degraded the relatively stable polymers such as cellulose, hemicellulose, and lignin. Bacteria degraded the more easily oxidized substances and the breakdown products of fungal activity that were not consumed by the fungi themselves. Fungal activity continued for as long as the organisms had access to air, but as they and their remaining substrate became buried and conditions became anaerobic, bacterial fermentation and anaerobic respiration, including methanogenesis, set in and continued until arrested by accumulation of inhibitory (toxic) wastes, lack of sufficient moisture, depletion of suitable electron acceptors for anaerobic respiration [i.e., nitrate, sulfate, Fe(III), and/or Mn(IV), carbon dioxide, etc.], and other factors (Francis, 1954; Kuznetsov et al., 1963; Rogoff et al., 1962). Combined with these limiting factors, the overwhelming accumulation of organic debris must also be taken into account. It was more than the system could handle before conditions for continued mineralization activity became unfavorable.

Uppermost aerobic layers of peat may sometimes harbor a viable microflora even today, indicating that peat formation may be occurring at the present time (Kuznetsov et al., 1963). Indeed, viable anaerobic bacteria and actinomycetes have even been detected in the deep layers of some peats (Rogoff et al., 1962; Zvyagintsev et al., 1993). Differences in methane production in various peats are a reflection of differences in their origin with respect to source materials and environmental conditions (e.g., Yavitt and Lang, 1990; Brown and Overend, 1993).

During its formation, peat becomes enriched in lignin, ulmins, and humic acids. The first of these compounds is a relatively stable polymer of woody tissue, and the second and third compounds are complex material from the incomplete breakdown of plant matter, including lignin. Peat also contains other compounds that are relatively resistant to microbial attack, such as resins and waxes from cuticles, stems, and spore exines of the peat-forming plants. Compared to the C, H, O, N, and S contents of the original undecomposed plant material, peat is slightly enriched in carbon, nitrogen, and sulfur, but depleted in oxygen and

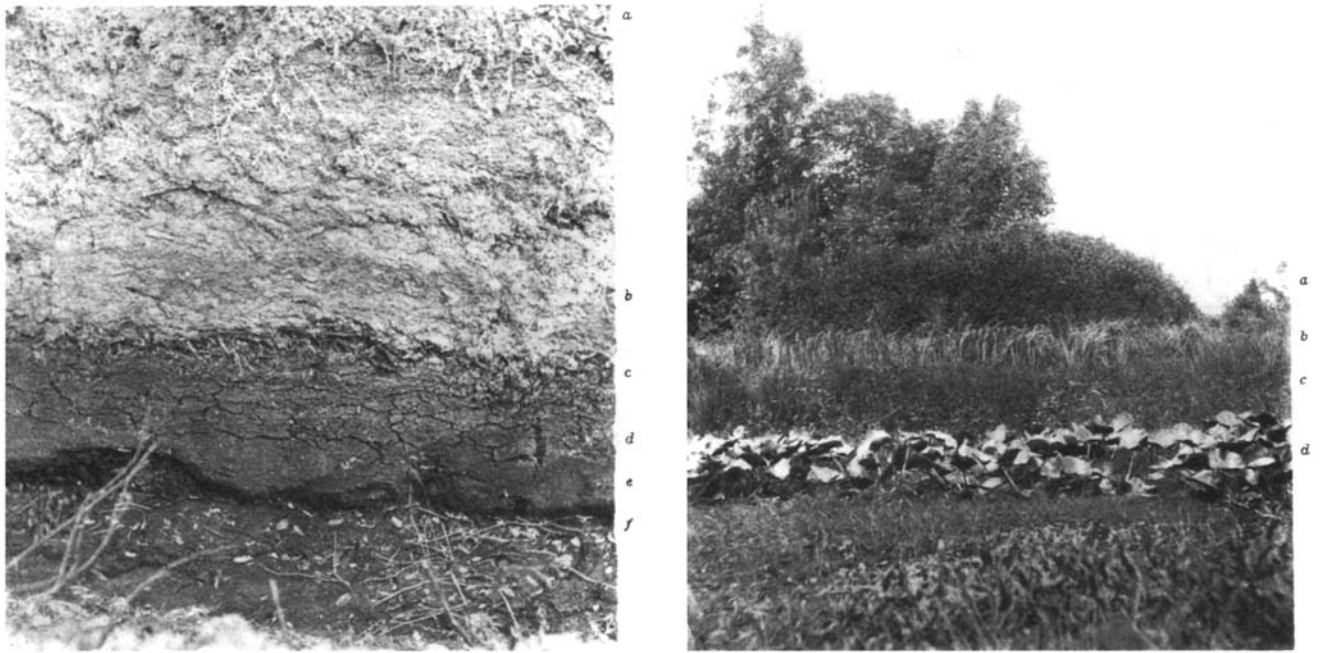


FIG. 21.8 Peat. (A) Section of ditch near Vestburg, showing light sphagnum peat over dark peat; a, living sphagnum; b, sphagnum peat, c, shrub remains, d, sedge rootstock, e, pond lily rootstocks, f, laminated peat. (B) View of surface near ditch, showing corresponding vegetative zones: a, shrub zone, b, grass zone, c, sedge zone, d, pond lily zone. (From Davis, 1907, Plate XII from Annual Report, Geological Survey of Michigan, with permission.)

sometimes hydrogen (Francis, 1954). This enrichment in C, N, and S over oxygen may be explained in part by the volatilization of products formed from the less resistant components by microbial attack and in part by the buildup of residues (resins, waxes, lignin) that have a relatively low oxygen content owing to their hydrocarbon-like and aromatic properties. The plant origin of peat is still clearly visible by examination of its structure.

21.5 COAL

The Nature of Coal

Coal has been defined by Francis (1954) as "a compact, stratified mass of mummified plants, which have been modified chemically in varying degrees, interspersed with smaller amounts of inorganic matter." Peat can be distinguished from coal in chemical terms by its much lower carbon content (51–59% dry wt) and higher hydrogen content (5.6–6.1% dry wt) compared to coal (carbon, 75–95% dry wt; hydrogen 2.0–5.8% dry wt) (Francis, 1954, p. 295). The average carbon content of typical wood has been given as 49.2% (dry wt), and its average hydrogen content as 6.1% (dry wt) (Francis, 1954). Coalification can thus be seen to have resulted in an enrichment in carbon and a slight depletion in hydrogen of the substance that gives rise to coal. Coal is generally found buried below layers of sedimentary stata (*overburden*). Its geologic age is generally advanced. Significant deposits formed in the Upper Paleozoic between 300 and 210 million years ago, in the Mesozoic between 180 and 100 million years ago, and in the Tertiary between 60 and 2.5 million years ago. Peats have generally developed from about 1 million years ago up to the present.

Coal is classified by rank. According to the ASTM classification system, four major classes are recognized (Bureau of Mines, 1965). They are, starting with the least developed coal, lignitic coal, subbituminous coal, and anthracite coal. Lignitic coal is the least developed coal, structurally resembling peat and having the highest moisture and lowest carbon content (59.5–72.3% dry wt) (Francis, 1954, p. 335) as well as having the lowest heat value of any of the coals. This coal formed in Tertiary times. Subbituminous coal has a somewhat lower moisture content and higher carbon content (72.3–80.4% dry wt). It also has a higher heat content than lignitic coal. Bituminous coal (Fig. 21.9) has a carbon content ranging from 80.4% to 90.9% (dry wt) and a high heat value. Both types of bituminous coal are mostly of Paleozoic and Mesozoic age. Anthracite coals have very low moisture content, few volatiles, and a high carbon content (92.9–94.7% dry wt). They are of Paleozoic age. Cannel coal is a special type of bituminous coal that was derived mainly from wind-blown spores and pollen rather than from woody plant tissue.

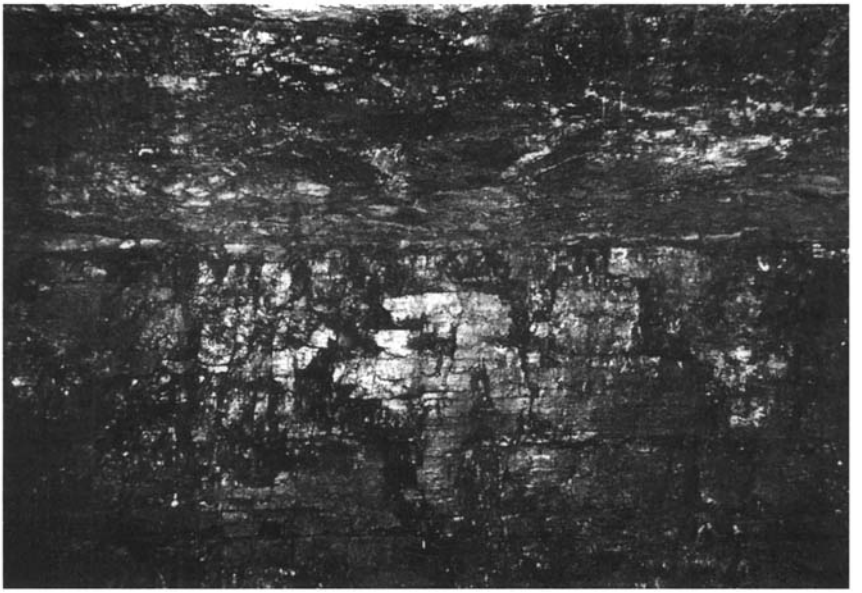


FIG. 21.9 A section of the Pittsburgh coal seam in the Safety Research coal mine of the U.S. Bureau of Mines, Pittsburgh, PA. Although not evident in this black-and-white photograph, extensive brown iron stains were present on portions of the face of the coal seam shown here. These stains are evidence of acid mine drainage emanating from the fracture at the upper limit of the coal seam. The acid drainage resulted from microbial oxidation of exposed iron pyrite inclusions in the seam (see text). (Courtesy of the U.S. Bureau of Mines.)

Role of Microbes in Coal Formation

As mentioned earlier, coal deposits developed at special periods in geologic time. In these periods, the climate, landscape features, and biological activity were favorable. Large amounts of plant debris accumulated in swamps or shallow lakes, because, owing to warm, moist climatic conditions, plant growth was very profuse and provided a continual supply of plant debris. At times the accumulated debris would become buried, covered by clay and sand under water, before a new layer of plant debris accumulated. As more sediment was deposited, subsidence followed and is believed to have played an integral part in the formation of coal deposits (Francis, 1954).

Bacteria and fungi are generally believed to have had an important role in coalification only in the initial stages. Their role was similar to that in peat formation. They destroyed the easily metabolized substances, such as sugars,

amino acids, and volatile acids, in a short time and degraded the more stable polymers, such as cellulose, hemicellulose, lignins, waxes, and resins more slowly. Many of the latter were degraded only very incompletely before microbial activity ceased for reasons similar to those in peat formation (see previous section). Hyphal remains, sclerotia, and fungal spores have been identified in some coal remains. Initial microbial attack is believed to have been aerobic and mainly fungal. Later attack, mostly by bacteria, occurred under progressively more anaerobic conditions. Tauson believed, however, that in coal formation the anaerobic phase was abiological (as cited by Kuznetsov et al., 1963, pp 79–81). Conversion of the residue from microbial activity (peat?) to coal is presently believed to have been due to physical and chemical agencies of an unidentified nature, but probably involved heat and pressure, which resulted in loss of volatile components.

Coal as a Microbial Substrate

Coal is not a very suitable nutrient to support microbial growth. According to early views, this is because coal contains inhibitory substances ("antibiotics") that may suppress it. These antibiotics have been thought to be associated with the waxy or resinous part of coal, extractable with methanol (Rogoff et al., 1962). In the first culture experiments with coal slurries, only marginal bacterial growth was obtainable, the limiting factors being the presence of inhibitory substances and lack of assimilable nutrients (Koburger, 1964). Growth of *Escherichia freundii* and *Pseudomonas rathonis* in such slurries improved when the coal was first treated with H_2O_2. In much more recent experiments with run-of-the-mine bituminous coal from Pennsylvania, in which coal particles in a size range of 0.5–13 mm were wetted in glass columns with air-saturated distilled water at 10- or 14-day intervals, a bacterial community did develop. It consisted mainly of autotrophic bacteria (iron and sulfur oxidizers) and to a lesser extent heterotrophic bacteria. Progressive acid production by the autotrophs was thought to limit the development of heterotrophs. Observed changes in the rate of acetate metabolism may be a reflection of microbial succession among the heterotrophs (Radway et al., 1987, 1989). The bacteria in these experiments lived at the expense of impurities in the coal, such as iron pyrite.

Two basidiomycete fungi, including *Trametes versicolor* (also known as *Polysporus versicolor* and *Coriolus versicolor*) and *Poria manticola*, have been shown to grow directly on crushed lignite coal as well as in minimal lignite–noble agar medium (Cohen and Gabriele, 1982). With time, the cultures growing on the lignite exuded a black liquid that was a product of lignite attack. Infrared spectra of the exudate gave an indication that conjugated aromatic rings from the lignite had been structurally modified. It must be stressed that the coal in this case was lignite (a low-rank coal) and not bituminous coal. Other fungi, including

Paecilomyces, *Penicillium* spp., *Phanerochaete chrysosporium*, *Candida* sp., and *Cunninghamella* sp., as well as *Streptomyces* sp. (actinomycete), have also been shown to grow on and degrade lignite and, in some cases, even bituminous coal (see Cohen et al., 1990; Stewart et al., 1990). In the case of *Trametes versicolor*, Cohen et al. (1987) and Pyne et al. (1987) at first attributed lignite solubilization to a protein secreted by the fungus that had polyphenol oxidase activity (syringaldazine oxidase) (Pyne et al., 1987). Subsequently Cohen et al. (1990) and Fredrickson et al. (1990) reported that a ligand produced by the fungus and identified as ammonium oxalate (Cohen et al., 1990) was the real solubilizing agent. The oxalate acted as a siderophore by removing iron from the test substrate leonardite (oxidized lignin). This later conclusion seems a little puzzling because it implies that iron(III) plays a central role in holding the leonardite structure together and that no modification of the organic skeleton of leonardite takes place.

White-rot fungi, of which *Polysporus versicolor* and *Phanerochaete chrysosporium* are examples, are known to produce two kinds of extracellular enzymes, lignin peroxidase and manganese-dependent peroxidase, both of which catalyze lignin attack (e.g., Paszczynski et al., 1986). Indeed, Stewart et al. (1990) reported that *P. chrysosporium* degraded lignite and a bituminous coal from Pennsylvania, albeit weakly. Some bacteria also can form such extracellular enzymes that are active on low-rank coals (Crawford and Gupta, 1993). Moreover, dibenzothiophene-degrading aerobic bacteria have been found that are able to break down part of the carbon framework of liquefied bituminous coal (suspension of pulverized coal) and in the process remove some of the sulfur bound in the framework (Stoner et al., 1990). It would therefore seem reasonable that such types of enzymes play a role in lignite attack. Depending on the coal, it is probably a combination of enzymatic and nonenzymatic processes that leads to transformation of the low-rank coals. The interested reader is referred to Crawford (1993) for further details on these processes.

Lignin and lignin derivatives can also be biologically attacked under anaerobic conditions (Young and Frazer, 1987). It would be of interest to study this action on lignite.

Microbial Desulfurization of Coal

Bituminous coal may contain significant amounts of sulfur in inorganic (pyrite, marcasite, elemental sulfur, sulfate) and/or organic form. The total sulfur can range from 0.5% to 11% (Finnerty and Robinson, 1986). The proportion of pyritic and organic sulfur in the total sulfur depends on the source of the coal. Iron pyrite or marcasite (FeS_2) came to be included in some bituminous coal seams during their formation. Some or all of this iron disulfide may well have been formed biogenically, in which case it is a reflection of the anaerobic biogenic phase of coalification, representative of sulfate respiration (see Chaps. 18 and 19)

and also the mineralization of organic sulfur compounds such as sulfur-containing amino acids. A model system illustrating how pyrite may have formed in coal is provided by the sulfur transformations presently occurring in the formation of Everglades peat (Casagrande and Siefert, 1977; Altschuler et al., 1983). The source of the sulfur in this case is organic.

The presence of pyritic sulfur in coal lowers its commercial value because on combustion of such coal, air pollutants such as SO_2 are generated. Pyritic sulfur can be removed in various ways (Bos and Kuenen, 1990; Blazquez et al., 1993). In all cases, the coal must first be pulverized to expose the pyrite. The pyrite particles can be separated by differential flotation in which pyrite flotation is suppressed. Flotation suppression can be achieved chemically or biologically. In the latter case, *Thiobacillus ferrooxidans*, which attaches rapidly and selectively to pyrite particles, is the suppression agent (Pooley and Atkins, 1983; Bagdigian and Myerson, 1986; Townsley et al., 1987). However, pyritic sulfur can also be removed by oxidizing it. This can be accomplished by the action of pyrite-oxidizing bacteria such as *Thiobacillus ferrooxidans*, *Sulfolobus* spp., *Acidianus* spp., and *Metallosphaera* (Dugan, 1986; Andrews et al., 1988; Merrettig et al., 1989; Larsson et al., 1990; Baldi et al., 1992; Clark et al., 1993).

Bituminous coal may also contain organically bound sulfur. Its presence is undesirable because on combustion it, too, contributes to air pollution. Microbiological methods are being sought to remove it, but progress has been slow (e.g., Dugan, 1986; Finnerty and Robinson, 1986; Crawford and Gupta, 1990; Mormile and Atlas, 1988; Van Afferden et al., 1990; Stoner et al., 1990; Omori et al., 1992; Olson et al., 1993; Izumi et al., 1994). Dibenzothiophene is used as a model structure for the organically bound sulfur in coal. Because the sulfur is likely to occur in different types of compounds bound in the structure of bituminious coal, whose degradation may require different enzyme systems, it may be that no one organism in nature can attack the whole range of these substances. Genetic engineering is being applied to find a solution to this problem. In any approach taken to remote organic sulfur from coal, it is important to find a new way to remove the sulfur without significant loss of carbon, which would lower the caloric value of the coal.

21.6 PETROLEUM

Nature of Petroleum

Petroleum is a mixture of aromatic and aliphatic hydrocarbons and various heterocyclics including oxygen-, nitrogen-, and sulfur-containing compounds. The aliphatic hydrocarbons include gaseous ones of the paraffinic series such as methane, ethane, propane, and butane, besides longer chain, nongaseous ones.

Some of the heterocyclic compounds, such as porphyrin derivatives, may contain metals such as vanadium or nickel bound in their structure. Petroleum accumulations are found in some folded, porous, sedimentary rock strata, such as limestone or sandstone, or in other fractured rock such as fissured shale or igneous rock. In petroleum geology these rock formations are collectively known as reservoir rock. The age of reservoir rocks may range from Late Cambrian (500 million years) to the Pliocene (1–13 million years). Very extensive petroleum reservoirs are found in rock of Tertiary age (70 million years) (North, 1985).

Most petroleum derived mainly from planktonic debris that was deposited on the floor of depressions of shallow seas and ultimately buried under heavy layers of sediment, deposited perhaps by turbidity currents. Over geologic time, the trapped organic matter became converted to petroleum and natural gas (chiefly methane) (North, 1985).

Many theories have been advanced to explain the origin of petroleum and associated natural gas (see, e.g., Beerstecher, 1954; Robertson, 1966; North, 1985). None of these have been fully accepted. Some theories invoke heat or pressure or both as agents that promoted abiological conversion of planktonic residues to the hydrocarbons and other constituents of petroleum and natural gas. The source of the heat has been viewed as the natural radioactivity of the Earth's interior but was more likely heat diffusing from magma chambers underlying tectonically active areas. Other theories have invoked inorganic catalysis with or without the influence of heat and pressure and with or without prior acid or alkaline hydrolysis. Still other theories have proposed that petroleum represents a residue of naturally occurring hydrocarbons in the planktonic remains after all other components have been biologically destroyed. It has even been proposed that biological agents produced the hydrocarbons by aerobic or anaerobic reduction of fatty acids, proteins, or amino acids, carbohydrates, carotenoids, sterols, glycerol, chlorophyll, and lignin–humus complexes, together with appropriate decarboxylations and deaminations. Finally, a theory has been put forward that methane, formed biogenically from the planktonic debris, became polymerized under high temperature and pressure in the possible presence of inorganic catalysts (see, e.g., Mango, 1992). Alternatively, it was theorized that bacteria modified the planktonic material to substances closely resembling petroleum components, which were then converted to petroleum and natural gas constituents by heat and pressure. Abiotically formed methane could also have been a source and could have been polymerized as proposed for biogenic methane. Chemical reaction of methane with liquid hydrocarbons has been noted in the laboratory at high temperature and pressure (1000 atm, 150–259°C) (Gold et al., 1986). At a hydrothermal mound area in the southern rift of the Guaymas Basin in the Gulf of California (Sea of Cortez), organic matter appears to be actively and abiotically transformed into petroliferous substances including gasoline-range aliphatic and aromatic hydrocarbons (Simoneit and Lonsdale, 1982; Didyk and Simoneit,

1989). This site may also be a source of methane used by methanotrophic consortia (see Sec. 21.3).

Role of Microbes in Petroleum Formation

At present it is generally thought that bacteria played a role in the initial stage of petroleum formation, but what this role was remains obscure, except in the case of methane formation. ZoBell (1952, 1953) suggested that the planktonic debris was fermented, leading to compounds enriched in hydrogen and depleted in oxygen, sulfur, and phosphorus. Davis (1967, p. 23) visualized microbial processes not unlike those in peat formation, involving aerobic attack of the sedimented planktonic debris followed by anaerobic activity after initial burial. This activity may have included hydrolytic, decarboxylating, deaminating, and sulfate-reducing reactions, resulting in the accumulation of marine humus, i.e., stabilized organic matter. Progressively deeper burial resulted in compaction and in cessation of microbial activity, accompanied by evolution of small amounts of hydrocarbon substance plus petroleum precursors. The biotic reactions were followed by an abiotic phase of longer duration during which the microbially produced precursors were transformed under the influence of heat and pressure into the range of hydrocarbons associated with petroleum. This sequence of biotic and abiotic reactions is supported by observations on light hydrocarbon formation in marine sediments (Hunt, 1984; Hunt et al., 1980). Clays could have catalytically promoted further chemical reductions of petroleum precursors.

Role of Microbes in Petroleum Migration in Reservoir Rock

As hydrocarbons accumulated during petroleum formation, the more volatile compounds generated increasing gas pressure, which helped force the more liquid components through porous rock (sandstone, limestone, fractured rock) to anticlinal folds. The hydrocarbons were trapped in the apex of these folds below a stratum of impervious rock to form a petroleum reservoir. We tap such reservoirs today for our petroleum supply. The migration of petroleum from the source rock to the reservoir rock was probably helped by groundwater movement and by the action of natural detergents such as fatty acid soaps and other surface-active compounds of microbial origin. ZoBell (1952) suggested that bacteria themselves may help to liberate oil from rock surfaces and thereby promote its migration by dissolving carbonate and sulfate minerals to which oil may adhere and by generating CO_2, whose gas pressure could help force the migration of petroleum. Bacterially produced methane may lower the viscosity of petroleum liquid by dissolving in it and thus help migration (ZoBell, 1952). The important microbial contributions to petroleum formation thus come in the initial

action on the source material (planktonic biomass) and in the final stages by promoting the migration of petroleum from the source rock to the reservoir rock. In addition, sulfate-reducing bacteria may play a role in sealing an oil deposit in reservoir rock by deposition of secondary $CaCO_3$ at the interface between the oil-bearing stratum and the stratal waters (Ashirov and Sazanova, 1962; Davis, 1967; Kuznetsov et al., 1963) (see also Chap. 8).

Viable bacteria have been detected in brines associated with petroleum reservoirs to which access was gained by drilling. They were assigned to three specific groups. One included the sulfate reducers *Desulfovibrio desulfuricans* and *Desulfotomaculum nigrificans* (Kuznetsov et al., 1963; Nazina and Rozanova, 1978). The second group included the methanogens *Methanobacterium mazei*, *Sarcina methanica* (now *Methanosarcina methanica*), and *Methanobacterium omelianskii* (now *Methanobacterium* MOH). The third group included the phototroph *Rhodopseudomonas palustris* (Rozanova, 1971), which is capable of anaerobic respiration in the dark (Madigan and Gest, 1978; Yen and Marrs, 1977). The petroleum-associated brines may be connate seawater whose mineral content was somewhat altered through contact with enclosing rock strata (see Chap. 5). Such brines may be low in sulfate but high in chlorides and not very conducive to microbial growth. Sulfate-containing groundwaters and alkaline carbonate waters, on mixing with the brines, can provide a milieu suitable for the activity of sulfate-reducing bacteria (Table 21.2). These waters furnish needed moisture and, in the case of sulfate-reducing bacteria, the terminal electron acceptor sulfate for their respiration. By current understanding of methanogenesis (see Sec. 21.3), the methane bacteria in these brines probably rely mainly on H_2 for energy and CO_2 as terminal electron acceptor and as the source of carbon. The sulfate-reducing bacteria may obtain their energy source directly from petroleum, as has been claimed by some, but may also depend on other bacteria to produce the compounds they need as carbon and energy sources (Ivanov, 1967) (Chaps. 18 and 19). If the plutonic waters associated with an oil reservoir are hydraulically connected with infiltrating surface waters, it is also possible that carbon and energy sources for the sulfate reducers derive at least in part from products produced by aerobic bacteria in oxidizing strata (Jobson et al., 1979). The observation of Panganiban and Hanson (1976) that at least one sulfate reducer

TABLE 21.2 Composition of Petroleum-Associated Brines (Percent Equivalents)

Cl^-	7.4–49.90	Ca^{2+}	0.33–11.02
SO_4^{2-}	0.03–10.06	Mg^{2+}	0.04–4.70
CO_3^{2-}	0.03–42.2	K^+ and Na^+	34.28–49.34

Source: After Kuznetsov et al. (1963), p. 17.

can use methane for energy and acetate for carbon makes a petroleum reservoir a not impossible direct source of an energy substrate for sulfate reducers. This notion is also supported by the recent discovery of a sulfate reducer that uses saturated hydrocarbon as an energy source (Aeckersberg et al., 1991).

Microbes in Secondary and Tertiary Oil Recovery

When a petroleum reservoir is first tapped for commercial exploitation, the initial oil is recovered by being forced to the surface by gas pressure from the volatile components of the oil and by pumping. This action, however, recovers only part of the total oil in a reservoir. To recover additional oil, a reservoir may be flooded with water by injection to force out additional oil (*secondary oil recovery*). Even secondary oil recovery will yield no more than 30–40% of the oil (North, 1985). To recover even a part of the remaining oil, which is more viscous than the previously extracted oil, *tertiary* or *enhanced oil recovery* treatment is necessary. This may involve a thermal method (e.g., steam injection) to reduce viscosity (e.g., North, 1985) or other chemical or physical methods (see, e.g., Orr and Taber, 1984). Alternatively, it may involve biological methods such as generation of surface-active agents of microbial origin to facilitate mobility of the oil or the generation of gas pressure by fermentation to force movement of the oil, or a combination of both processes (McInerney and Westlake, 1990; Tanner et al., 1991; Adkins et al., 1992). Microbially enhanced oil recovery (MEOR) may also involve the promotion of selective plugging by microbes of high permeability zones in oil reservoirs to increase volumetric sweep efficiency and the microscopic oil displacement efficiency (Raiders et al., 1989; McInerney and Westlake, 1990). Volumetric sweep efficiency refers to the ability of injected water to recover oil from the less permeable zones.

Water injection into oil reservoirs stimulates microbial activity by sulfate reducers as well as methanogens and fermenting bacteria (Rozanova, 1978; Nazina et al., 1985; Belyaev et al., 1990a, 1990b). Because the injected water carries oxygen, hydrocarbon-oxidizing bacteria have also been detected (Belyaev et al., 1990a, 1990b). Indeed, a succession of organisms may occur, with the aerobic hydrocarbon oxidizers and others producing the substrates (e.g., acetate and higher fatty acids) for fermenting and sulfate-reducing bacteria, and the fermenting bacteria producing hydrogen for use by methanogens and sulfate reducers (Nazina et al., 1985). In the Bondyuzh oil field (former Tatar SSR), maximal numbers of aerobic hydrocarbon-oxidizing bacteria were found at the interface of injection and stratal waters. The destructive effect of these bacteria on petroleum, in one case in which this was studied, appeared to be limited by the salt concentration of the stratal waters (Gorlatov and Belyaev, 1984).

For tertiary oil recovery that involves use of surface-active agents of microbial origin, xanthan gums of the bacterium *Xanthomonas campestris* or

glucan polymers of fungi such as *Sclerotium*, *Stromantinia*, and *Helotium* (Compere and Griffith, 1978) may be generated in separate processes and then injected into an oil well to facilitate oil recovery. As an alternative approach, appropriate organisms may be introduced into the oil well to produce a surface-active agent in situ (Finnerty et al., 1984). Promotion of tertiary oil recovery may also involve injection of dilute molasses solution into an oil well and subsequent injection of a gas-producing culture such as *Clostridium acetobutylicum*, which ferments the molasses to the solvents acetone, butanol, and ethanol and large amounts of CO_2 and H_2. The solvents help to lower the viscosity of the oil, and the gases provide pressure to move the oil (Yarbrough and Coty, 1983).

Removal of Organic Sulfur from Petroleum

As in the case of coal, petroleum that contains significant amounts of organic sulfur may not be usable as fuel because of air pollution by the volatile sulfur compounds such as SO_2 that result from its combustion. The feasibility of removing this sulfur microbiologically has been actively explored (e.g., Foght et al., 1990). The experimental approach being taken is similar to that in the investigations to remove organic sulfur from coal. The model substance to evaluate microbial desulfurizing activity is dibenzothiophene. The best microbial agents for any industrially applicable process are those that remove the sulfur without significant concomitant oxidation of the carbon to CO_2. *Rhodococcus erythropolis* is an example of such an organism (e.g., Izumi et al., 1994), and *Rhodococcus* sp. strain ECRD-1 (Grossman et al., 2001) is another.

Microbes in Petroleum Degradation

When natural petroleum reservoirs become industrially exploited, constituents in the oil, whether the oil is still in its reservoir or removed from it, become susceptible to microbial attack. This attack may be aerobic or anaerobic. An extensive literature has built up around this subject, largely because such microbial attack can be used in the management of oil pollution. Only the major principles of microbial petroleum degradation will be discussed here.

Although hydrocarbon oxidation was once considered a strictly aerobic process because the initial attack usually involves an oxygenation, clear evidence now exists as well for anaerobic degradation of some oil constituents. Old claims, to which reference has already been made, that sulfate-reducing bacteria in oil well brines are able to derive energy and/or carbon from petroleum constituents, especially methane, can be found in the literature (Davis, 1967, p. 243; Davis and Yarbrough, 1966; Panganiban et al., 1979) (see also Sec. 21.3). It was also suggested that even if sulfate reducers were not able to attack hydrocarbons themselves, satellite organisms might be able to convert such compounds to

products that could be used by the sulfate reducers (Dutova, 1962). Kuznetsova and Gorlenko (1965) reported anaerobic attack of hydrocarbons by a strain of *Pseudomonas* in a mineral salts medium with petroleum as the only carbon source. The bacterial population in these experiments increased a millionfold maximally; the redox potential dropped from +40 to −110 mV. Similarly, Kvasnikov et al. (1973) obtained growth of *Clostridium* (*Bacillus*) *polymyxa* anaerobically with *n*-alkanes as the sole source of carbon. Simakova et al. (1968) found that methane and high-paraffinaceous oil were more intensely attacked aerobically than anaerobically. They detected very similar products, including fatty acids of high and low molecular weight, amino acids, alcohols and aldehydes, under either condition. However, hydroxyacids were formed only aerobically. This work showed that petroleum degradation is much slower anaerobically than aerobically.

Ward and Brock (1978) observed very slow anaerobic conversion of [1-^{14}C]hexadecane added to reducing sediments and bottom water from Lake Mendota, Wisconsin. They reported that 13.7% of the [1-^{14}C]hexadecane in the sediment was converted to $^{14}CO_2$ and ^{14}C-containing cell carbon in 375 hr of incubation. Aerobically the hexadecane was degraded much more rapidly in the same sediment samples. Zehnder and Brock (1979) found that methanogens are able to oxidize small amounts of the methane they form anaerobically. The methane oxidation mechanism in these organisms seems to differ from the methane-forming mechanism. The slow rate of anaerobic hydrocarbon degradation, when it occurs, helps to explain in part why petroleum has remained preserved over eons of time. However, prolonged periods of an absence of degradative activity of any kind must have been a more important factor in its preservation. Nevertheless, a case of in situ microbial conversion of petroleum into bitumen has been attributed to Alberta (Canada) oil sands, based on laboratory simulation (Rubinstein et al., 1977).

Current State of Knowledge of Aerobic and Anaerobic Petroleum Degradation by Microbes

A variety of bacteria and fungi are able to metabolize hydrocarbons (Atlas, 1981, 1984, 1988; Atlas and Bartha, 1998). Some examples are listed in Table 21.3. The mode of attack of hydrocarbons by microorganisms depends on the kind of organism involved and the environmental conditions. Aerobically, alkanes may be attacked monoterminally to form an alcohol by an oxygenation (Doelle, 1975; Atlas, 1981; Gottschalk, 1986):

$$\begin{aligned} RCH_2CH_3 &\xrightarrow{+0.5O_2} RCH_2CH_2OH \xrightarrow{-2H} RCH_2CHO \\ &\xrightarrow{+H_2O,-2H} RCH_2COOH \end{aligned} \qquad (21.16)$$

TABLE 21.3 Microorganisms Capable of Aerobic Hydrocarbon Metabolism

Organism	Substrate	Mode of attack	Reference[a]
Pseudomonas oleovorans	Octane	Desaturation	Abbott and Hou, 1973
P. fluorescens, P. aeruginosa	Aromatic hydrocarbons	Oxidation	Van der Linden and Thijsse, 1965
Nocardia salmonicolor	Hexadecane	Desaturation	Abbott and Casida, 1968
Yeasts		NS[b]	Ahearn et al., 1971
Trichosporon	*n*-Paraffins	NS	Barna et al., 1970
Arthrobacter	*n*-Alkane aromatics	Oxidation Oxidation	Klein et al., 1968; Stevenson, 1967
Mycobacterium	Butane	NS Oxidation	Nette et al., 1965; Phillips and Perry, 1974
Brevibacterium erythrogenes	Alkane	Oxidation	Pirnik et al., 1974
Nocardia	Mono- and dicyclic hydrocarbons	Oxidation	Raymond et al., 1967
Cladosporium	*n*-Alkane	NS Oxidation	Teh and Lee, 1973; Walker and Cooney, 1973
Graphium	Ethane	Oxidation	Volesky and Zajic, 1970

[a] For recent reviews, see Assinder and Williams (1990), Cerniglia (1984), and Atlas (1984).
[b] NS, not specified.

This is followed sequentially by oxidation to an aldehyde and then a corresponding carboxylic acid [reaction (21.16)]. The carboxylic acid is oxidized to acetate, which is then oxidized to CO_2 and H_2O.

Alkanes may also be monoterminally attacked to form a ketone (Fredericks, 1967) or a hydroperoxide (Stewart et al., 1959). They may also be attacked diterminally (Doelle, 1975). For instance, *Pseudomonas aeruginosa* can attack 2-methylhexane at either end of the carbon chain, forming a mixture of 5-methylhexanoic and 2-methylhexanoic acid (Foster, 1962). Furthermore, alkanes may be desaturated terminally or subterminally, forming alkenes (Chouteau et al., 1962; Abbott and Casida, 1968). Subterminal desaturation may proceed as follows:

$$\text{Hexadecane} \longrightarrow \begin{matrix}\text{8-hexadecene}\\ \text{7-hexadecene}\\ \text{6-hexadecene}\end{matrix} \qquad (21.17)$$

Alkenes may be attacked by forming epoxides, which may then be further metabolized (Abbott and Hou, 1973); diols may be formed in the process. In all

the foregoing processes atmospheric oxygen acts as terminal electron acceptor and as the source of oxygen in oxygenation.

Until very recently, anaerobic attack of alkanes and alkenes was believed possible only if these compounds carried one or more substituents, in particular halogens. Dichloromethane (CH_2Cl_2) can be degraded anaerobically. In these reactions, the initial attack cannot involve oxygenation but instead involves a dechlorination step. In the case of dichloromethane, the proposed reaction carried out by a consortium of two different bacterial strains (Braus-Stromeyer et al., 1993) is summarized as

$$CH_2Cl_2 + H_2O \rightarrow (HCHO) + 2HCl \tag{21.18}$$

This reaction is followed by an oxidation of the formaldehyde-like intermediate (HCHO) to formic acid. The formic acid is subsequently converted to acetate in an acetogenic reaction (Braus-Stromeyer et al., 1993).

In the case of tetra- and trichloroethylene, anaerobic dechlorination by a reductive process has been observed. In the process, the chlorinated hydrocarbon serves as terminal electron acceptor. Complete bacterial dechlorination of tetra- and trichloroethylene to ethylene was observed by Freedman and Gossett (1989), Ensley (1991), and Maymó-Gatell et al. (1999). De Bruin et al. (1992) observed the formation of ethane by reductive transformation of tetrachloroethene.

A clear demonstration that a saturated, unsubstituted alkane can be mineralized by anaerobic bacteria was presented by Aeckersberg et al. (1991). These investigators isolated a sulfate-reducing bacterium, strain HxD3, from the precipitate of an oil–water separator in an oil field near Hamburg, Germany. This organism mineralized hexadecane using sulfate as oxidant (terminal electron acceptor). The nature of the initial attack of the hexadecane was not elucidated. The overall reaction of hexadecane mineralization was consistent with the following stoichiometry (Aeckersberg et al., 1991):

$$C_{16}H_{34} + 12.25SO_4^{2-} + 8.5H^+ \rightarrow 16HCO_3^- + 12.25H_2S + H_2O \tag{21.19}$$

Ehrenreich et al. (2000) demonstrated that three distinct types of denitrifying bacteria were able to oxidize alkanes anaerobically.

A wide range of aromatic compounds can be aerobically and anaerobically degraded by microbes (Tables 21.3 and 21.4). *Aerobic scission* of the ring structure of the aromatic compounds involves oxygenation either between adjacent oxygenated carbon atoms (ortho fission) or adjacent to one of them (meta fission) (Dagley, 1975). In the case of benzene, aerobic degradation of the ring structure involves an initial hydroxylation catalyzed by a mixed function or monooxygenase to form catechol followed by action of dioxygenase to cleave the catechol ring to form *cis,cis*-muconate by ortho fission. This product can then be degraded enzymatically in several steps to acetate (Doelle, 1975). The acetate is

TABLE 21.4 Bacteria Capable of Anaerobic Metabolism of Aromatic and Heterocyclic Hydrocarbons

Organism	Substrate	Mode of attack	Reference[a]
Rhodopseudomonas palustris	Benzoate, hydroxybenzoate	Reductive ring cleavage	Dutton and Evans, 1969
Desulfobacterium phenolicum	Phenol and derivatives	Anaerobic degradation	Bak and Widdel, 1986a
Desulfobacterium indolicum	Indolic compounds	Anaerobic degradation	Bak and Widdel, 1986b
Desulfobacterium catecholicum	Catechol	Anaerobic degradation	Szewzyk and Pfennig, 1987
Desulfococcus niacini	Nicotinic acid	Anaerobic degradation	Imhoff-Stuckle and Pfennig, 1983

[a] For reviews, see Evans and Fuchs (1988), Reineke and Knackmuss (1988), and Higson (1992).

then oxidized to CO_2 and H_2O. Naphthalene, anthracene, and phenanthrene and derivatives can be degraded by a similar mechanism by attacking each ring in succession (Doelle, 1975). In some instances, benzene derivatives are attacked by meta fission instead of ortho fission as in the previous examples (Doelle, 1975). *Anaerobic scission* of the ring structure involves ring saturation, hydration, and dehydrogenation (Evans and Fuchs, 1988; Colberg, 1990; Grbic-Galic, 1990). Some aromatic hydrocarbons such as benzoate can also be biodegraded anaerobically by photometabolism of certain Rhodospirillaceae (purple nonsulfur bacteria) (Table 21.4). Ring cleavage is by hydration of pimelate (Dutton and Evans, 1969).

Unlike chorinated alkanes, chlorinated aromatics have been shown to be completely degradable anaerobically, by a consortium of bacteria (e.g., Sharak Genthner et al., 1989; Colberg, 1990; Grbic-Galic, 1990). In one kind of consortium, *Desulfomonile tiedjei* DCB-1 acts on a chlorinated hydrocarbon by using it as terminal electron acceptor in a respiratory process that includes dechlorination (Shelton and Tiedje, 1984; Dolfing, 1990; Mohn and Tiedje, 1990, 1991). Subsequent mineralization of the dechlorinated aromatic product depends on other anaerobic organisms in the consortium.

The ability of an organism to attack hydrocarbons aerobically does not necessarily mean that it can use such a compound as the sole source of carbon and energy. Many cases are known in which hydrocarbons are oxidized in a process known as **co-oxidation**, wherein another compound, which may be quite unrelated, is the carbon and energy source but which somehow permits the simultaneous oxidation of the hydrocarbon. Examples are the oxidation of ethane

to acetic acid, the oxidation of propane to propionic acid and acetone, and the oxidation of butane to butanoic acid and methyl ethyl ketone by *Pseudomonas methanica* growing on methane, as first shown by Leadbetter and Foster (1959). Methane is the only hydrocarbon on which this organism can grow. Another example is the oxidation of alkylbenzenes by a strain of *Micrococcus cerificans* growing on *n*-paraffins (Donos and Frankenfeld, 1968). Still other examples have been summarized by Horvath (1972).

Chain length and branching of aliphatic hydrocarbons can affect microbial attack. In situ observations revealed rapid microbial degradation of pristane and phytane (Atlas and Cerniglia, 1995). Some bacteria that attack alkanes of chain lengths C_8–C_{20} may not be able to attack alkanes of chain lengths C_1–C_6, whereas others cannot grow on alkanes of chain lengths greater than C_{10} (Johnson, 1964). Fungi are known that can grow on alkanes of chain lengths up to C_{34}. It has also been noted that certain placements of methyl or propyl groups in the alkane carbon chain lessen or prevent utilization of the compounds (McKenna and Kallio, 1964).

Use of Microbes in Prospecting for Petroleum

Prospecting for petroleum through detection of hydrocarbon-utilizing microorganisms has been proposed. The basis for this method is detection of microseepage of petroleum or some of its constituents, especially the more volatile components, in the ground overlying a deposit using the presence of the hydrocarbon-utilizing microorganisms as indicators. It involves enriching soil, sediment, and water samples from a suspected seepage area for microbes that can metabolize gaseous hydrocarbons and demonstrating hydrocarbon consumption (Davis, 1967). An enrichment medium consisting of a mineral salts solution with added volatile hydrocarbon (ethane, propane, butane, isobutane) is satisfactory. Methane-oxidizing bacteria are poor indicators in petroleum prospecting because methane can occur in the absence of petroleum deposits and, moreover, some methane-oxidizing bacteria are unable to oxidize other aliphatic hydrocarbons. Detection of bacteria that can oxidize ethane and longer chain hydrocarbons, on the other hand, provides presumptive evidence for a hydrocarbon seep and an underlying petroleum reservoir (Davis, 1967). It is assumed that ethane and propane formed in anaerobic fermentation are produced in quantities too small to select for a hydrocarbon-utilizing microflora. Likely organisms active in soil enrichments from hydrocarbon seeps may include *Mycobacterium paraffinicum* and *Streptomyces* spp.

Hydrocarbon enrichment cultures may be prepared using ^{14}C-labeled hydrocarbon. This allows easy quantification of the activity of hydrocarbon-oxidizing bacteria in water and sediments (Caparello and LaRock, 1975). With

this method, the hydrocarbon-oxidizing potential of a sample can be correlated with the hydrocarbon burden of the environment from which the sample came.

Microbes and Shale Oil

North (1985) described *oil shales* as either bituminous, nonmarine limestones, or marlstones containing kerogen. *Tar sands* are consolidated or unconsolidated rock coated with bituminous material (North, 1985). *Bitumens* are solid hydrocarbons that are soluble in organic solvents and fusible below ~150°C. *Kerogens* are insoluble in organic solvents. They are intermediate products in the diagenetic transformation of organic matter in sediments and are considered a precursor in petroleum formation. As in the formation of peat, coal, and petroleum, fungi and bacteria probably played a role in the early stages of transformation of the source material (mostly terrestrial microbial biomass). Later stages involved physicochemical processes. However, in the case of oil sand bitumens of Alberta, the origin appears to be partial biodegradation of petroleum leaving behind the high-viscosity components (Rubinstein et al., 1977).

Bitumen and kerogen can be converted to a petroleum-like substance by heat treatment (e.g., retorting) (North, 1985). Separation from host rock, especially if it is limestone, can be facilitated if the limestone is dissolved. This can be achieved, at least on a laboratory scale, by acid formed by microbes (e.g., sulfuric acid from S^0 oxidation by *Thiobacillus thiooxidans*) (Meyer and Yen, 1976).

Although raw shale oil is considered relatively resistant to microbial attack, some reports indicate otherwise. Both aerobic and anaerobic attack have been observed, but the anaerobic attack proceeded at a much slower rate (Roffey and Norqvist, 1991; Wolf and Bachofen, 1991; Ait-Langomazino et al., 1991). Hydrogenated shale oil was found to be readily metabolized by some gram-negative organisms (*Alcaligenes* and *Pseudomonas* or *Pseudomonas*-like organisms) (Westlake et al., 1976).

21.7 SUMMARY

Not all carbon in the biosphere is continually being recycled. Some is trapped in special sedimentary formations, where it is inaccessible to microbial attack. The forms in which the trapped carbon appears are methane, peat, coal, petroleum, bitumen, and kerogen.

Most methane in sedimentary formations is of biogenic origin. It may occur by itself or in association with coal or petroleum deposits. Its biogenic formation is a strictly anaerobic process involving methanogenic bacteria that may reduce CO_2, formate, methanol, methylamines, or acetate with H_2 or transform acetate to methane and CO_2 in the absence of H_2. The autotrophic methanogens use a

unique mechanism for CO_2 assimilation that involves reduction of one CO_2 to methyl carbon and a second CO_2 to a formyl carbon and then coupling the two to form acetate. The acetate is subsequently carboxylated to form pyruvate, the key intermediate for forming building blocks for all the cell constituents. Methanogenesis can occur mesophilically and thermophilically.

Methane may be oxidized and assimilated by a special group of microorganisms called methanotrophs. This process is generally aerobic, although evidence of anaerobic methane oxidation exists. At least one of the organisms responsible for anaerobic methane consumption is a sulfate reducer. Limited anaerobic methane oxidation by a methanogen has also been observed. Carbon assimilation by aerobic methanotrophs may be via a hexulose monophosphate pathway or the serine pathway, in each case involving integration of the carbon at the oxidation level of formaldehyde that is produced as an intermediate in methane oxidation. In addition, both types of methanotrophs derive some of their carbon from CO_2.

Peat is the result of partial biodegradation of plant remains accumulating in marshes and bogs. Aerobic attack by enzymes in the plant debris and by fungi and some bacteria initiates the process. It is followed by anaerobic attack by bacteria during burial resulting from continual sedimentation until inhibited by accumulating wastes, lack of sufficient moisture, and so on. A viable microbial flora can usually be detected in peat even though the peat may have formed over a geologically extended period. Coal is thought to have formed like peat, except that in the advanced stages, as a result of deeper burial, it was subject to physical and chemical influences that converted the peat to coal. Different ranks of coal exist, which differ from each other largely in carbon content and heat value. It is questionable whether coal itself harbors an indigenous flora. Bituminous coal has pyrite or marcasite associated with it. Upon exposure to air and moisture during mining, this iron disulfide becomes subject to attack by acidophilic, iron-oxidizing thiobacilli and is the source of acid mine drainage.

Whereas peat and coal derived from terrestrial plant matter, petroleum and associated natural gas (mostly methane) are derived from phytoplankton remains that accumulated in depressions of shallow seas and became gradually buried. Microbial attack altered these remains biochemically until complete burial by accumulating sediment stopped the organisms. In tectonically active areas, the buried and biochemically altered organic matter became subject to further alteration by heat from magmatic activity and pressure from overlying sediment. These chemical alterations may have been catalyzed by clay minerals. The final products of these transformations were petroleum hydrocarbons. At least some of the natural gas associated with petroleum may represent biogenic methane formed in the initial stages of plankton debris fermentation. At its site of formation, petroleum is highly dispersed. As a result of gas (natural gas, CO_2) and hydrostatic pressure as well as lubrication of the surfaces of the sediment

matrix by bacteria and some of their products, matured petroleum may be forced to migrate through pervious sediment strata until it is caught in a trap such as an anticlinal fold. It is such petroleum-filled traps that constitute commercially exploitable petroleum reservoirs. Sulfate-reducing bacteria may assist in trapping petroleum by laying down impervious calcite layers. This calcite may, however, also interfere with petroleum recovery.

At least some petroleum hydrocarbons can be oxidized in air by certain bacteria and fungi. Anaerobic bacterial attack of unsubstituted alkanes as well as chlorinated alkanes and of a wide range of aromatic compounds has also been demonstrated. Hydrocarbon-utilizing microorganisms may be used as indicators in prospecting for petroleum.

REFERENCES

Abbott BJ, Casida LE Jr. 1968. Oxidation of alkanes to internal monoalkenes by a Nocardia. J Bacteriol 96:925–930.

Abbott BJ, Hou CT. 1973. Oxidation of 1-alkanes to 1,2epoxy-alkanes by *Pseudomonas oleovorans*. Appl Microbiol 26:86–91.

Abrams JW, Nedwell DB. 1978. Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen. Arch Microbiol 117:89–92.

Adkins JP, Tanner RS, Udegbunam EO, McInerney MJ, Knapp RM. 1992. Microbially enhanced oil recovery from unconsolidated limestone cores. Geomicrobiol J 10:77–86.

Aeckersberg F, Bak F, Widdel F. 1991. Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. Arch Microbiol 156:5–14.

Ahearn DG, Meyers SP, Standard PG. 1971. The role of yeasts in the decomposition of oils in marine environments. Dev Ind Microbiol 12:126–134.

Ait-Langomazino N, Sellier R, Jouquet G, Trescinski M. 1991. Microbial degradation of bitumen. Experientia 47:533–539.

Alexander M. 1977. Introduction to Soil Microbiology. 2nd ed. New York: Wiley.

Altschuler ZS, Schnepfe MM, Silber CC, Simon FO. 1983. Sulfur diagenesis in Everglades peat and origin of pyrite in coal. Science 221:221–227.

Andrews G, Darroch M, Hansson T. 1988. Bacterial removal of pyrite from concentrated coal slurries. Biotech Bioeng 32:813–820.

Anthony C. 1986. Bacterial oxidation of methane and methanol. Adv Microb Physiol 27:113–210.

Ashirov KB, Sazanova IV. 1962. Biogenic sealing of oil deposits in carbonate reservoirs. Mikrobiologiya 31:680–683 (Engl transl, pp 555–557).

Assinder SJ, Williams PA. 1990. The TOL plasmids: Determinants of the catabolism of toluene and the xylenes. Adv Microb Physiol 31:1–69.

Atlas RM. 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiol Rev 45:180–209.

Atlas RM. 1984. Petroleum Microbiology. New York: McGraw-Hill.

Atlas RM. 1988. Microbiology. Fundamental and Applications. 2nd ed. New York: Macmillan.

Atlas RM. 1997. Principles of Microbiology. 2nd ed. Boston, MA: WCB McGraw-Hill.
Atlas RM, Bartha R. 1998. Microbial Ecology: Fundamentals and Applications, 4th ed. Menlo Park, CA: Benjamin Cummings.
Atlas RM, Cerniglia CE. 1995. Bioremediation of petroleum pollutants. BioScience 45:332–338.
Bagdigian RM, Myerson AS. 1986. The adsorption of *Thiobacillus ferrooxidans* on coal surfaces. Biotech Bioeng 28:467–479.
Bak F, Widdel F. 1986a. Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum* sp. nov. Arch Microbiol 146:177–180.
Bak F, Widdel F. 1986b. Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., sp. nov. Arch Microbiol 46:170–176.
Baldi F, Clark T, Pollack SS, Olson GJ. 1992. Leaching pyrites of various reactivities by *Thiobacillus ferrooxidans*. Appl Environ Microbiol. 58:1853–1856.
Barna PK, Bhagat SD, Pillai KR, Singh HD, Branah JN, Iyengar MS. 1970. Comparative utilization of paraffins by a *Trichosporon* species. Appl Microbiol 20:657–661.
Beerstecher E. 1954. Petroleum Microbiology. Houston, TX: Elsevier.
Belyaev SS, Rozanova EP, Borzenkov IA, Charakhch'ykan IA, Miller YuM, Sokolov MYu, Ivanov MV. 1990a. Characteristics of microbiological processes in a water-flooded oilfield in the middle Ob' region. Microbiologiya 59:1075–1081 (Engl transl, pp 754–759).
Belyaev SS, Borzenkov IA, Milekhina EI, Charakhch'yan IA, Ivanov MV. 1990b. Development of microbiological processes in reservoirs of the Romashkino oilfield. Mikrobiologiya 59:1118–1126 (Engl transl, pp 786–792).
Bhatnagar L, Jain MK, Zeikus JG. 1991. Methanogenic bacteria. In: Shivley JM, Barton LL, eds. Variations in Autotrophic Life. London, UK: Academic Press, pp 251–270.
Blaut M, Gottschalk G. 1984. Protonmotive force-driven synthesis of ATP during methane formation from molecular hydrogen and formaldehyde or carbon dioxide in *Methanosarcina barkeri*. FEMS Microbiol Lett 24:103–107.
Blaut M, Peinemann S, Deppenmeier U, Gottschalk G. 1990. Energy transduction in vesicles of methanogenic strain Gö 1. FEMS Microbiol Rev 87:367–372.
Blaut M, Müller V, Gottschalk G. 1992. Energetics of methanogenesis studied in vesicular systems. J Bioenerg Biomembr 24:529–546.
Blazquez ML, Ballester A, Gonzalez F, Mier JL. 1993. Coal biodesulfurization. Bio-recovery 2:155–157.
Bochem HP, Schoberth SM, Sprey B, Wengler P. 1982. Thermophilic biomethanation of acetic acid: Morphology and ultrastructure of granular consortium. Can J Microbiol 28:500–510.
Bock A-K, Prieger-Kraft A, Schönheit P. 1994. Pyruvate:A novel substrate for growth and methane formation in *Methanosarcina barkeri*. Arch Microbiol 161:33–46.
Boone DR, Whitman WB, Rouvière P. 1993. Diversity and taxonomy of methanogens. In: Ferry JG, ed. Methanogenesis. New York: Chapman and Hall, pp 35–89.
Bos P, Kuenen JG. 1990. Microbial treatment of coal. In: Ehrlich HL, Brierley CL, eds. Microbial Mineral Recovery. New York: McGraw-Hill, pp 343–377.
Bowen HJM. 1979. Environmental Chemistry of the Elements. London, UK: Academic Press.

Braus-Stromeier SA, Hermann R, Cook AM, Leisinger T. 1993. Dichloromethane as the sole carbon source for an acetogenic mixed culture and isolation of a fermentative, dichloromethane-degrading bacterium. Appl Environ Microbiol 59:3790–3797.

Breznak JA. 1982. Intestinal microbiota of termites and other xylophagus insects. Annu Rev Microbiol 36:323–343.

Brock TD, Madigan MT. 1988. Biology of Microorganisms. 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Brooks JM, Kennicutt MC II, Fisher CR, Macko SA, Cole K, Childress JJ, Bridigare RR, Vetter RD. 1987. Deep-sea hydrocarbon seep communities: Evidence for energy and nutritional carbon sources. Science 238:1138–1142.

Brown DA, Overend RP. 1993. Methane metabolism in raised bogs of northern wetlands. Geomicrobiol J 11:35–48.

Bryant MP, Campbell LL, Reddy CA, Crabill MR. 1977. Growth of *Desulfovibrio* in lactate or methanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl Environ Microbiol 33:1162–1169.

Bureau of Mines. 1965. Mineral Facts and Problems. Bull 630. Washington, DC: Bur Mines, US Dept of the Interior.

Caparello DM, LaRock PA. 1975. A radioisotope assay for quantification of hydrocarbon biodegradation potential in environmental samples. Microb Ecol 2:28–42.

Casagrande D, Siefert K. 1977. Origins and sulfur in coal: Importance of the ester sulfate content of peat. Science 195:675–676.

Cavanaugh CM, Levering PR, Maki JS, Mitchell R, Lidstron ME. 1987. Symbiosis of methylotrophic bacteria and deep-sea mussels. Nature (Lond) 325:346–348.

Cerniglia CE. 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl Microbiol 30:31–71.

Chen W, Konisky J. 1993. Characterization of a membrane-associated ATPase from *Methanococcus voltae*, a methanogenic member of the Archaea. J Bacteriol 175:5677–5682.

Childress JJ, Fisher CR, Brooks JM, Kennicutt MC II, Bidigare R, Anderson AE. 1986. A methanotarophic marine molluscan (Bivalvia, Mytilidae) symbiosis: Mussel fueled by gas. Science 233:1306–1308.

Chouteau J, Azoulay E, Senez JC. 1962. Dégradation bactérienne des hydrocarbons paraffiniques. IV. Identification par spectrophotométrie infrarouge du hept-1-ene produit à partir du n-heptane par des suspensions nonproliférantes de *Pseudomonas aeruginosa*. Bull Soc Chim Biol 44:1670–1672.

Clark TR, Baldi F, Olson GJ. 1993. Coal depyritization by the thermophilic archeon *Metallosphaera sedula*. Appl Environ Microbiol 59:2375–2379.

Cohen MS, Gabriele PD. 1982. Degradation of coal by the fungi *Polysporus versicolor* and *Poria monticola*. Appl Environ Microbiol 44:23–27.

Cohen MS, Bowers WC, Aronson H, Gray ET Jr. 1987. Cell-free solubilization of coal by *Polysporus versicolor*. Appl Environ Microbiol 53:2840–2843.

Cohen MS, Feldman KA, Brown CS, Gray ET Jr. 1990. Isolation and identification of the coal-solubilizing agent produced by *Trametes versicolor*. Appl Environ Microbiol 56:3285–3291.

Colberg PJS. 1990. Role of sulfate in microbial transformations of environmental contaminants: Chlorinated aromatic compounds. Geomicrobiol J 8:147–165.

Compere AL, Griffith WL. 1978. Production of high viscosity glucans from hydrolyzed cellulosics. Dev Ind Microbiol 19:601–607.
Crawford DL, ed. 1993. Microbial Transformations of Low Rank Coals. Boca Raton, FL: CRC Press.
Crawford DL, Gupta RK. 1990. Oxidation of dibenzothiophene by *Cunninghamella elegans*. Curr Microbiol 21:229–231.
Crawford DL, Gupta RK. 1993. Microbial depolymerization of coal. In: Crawford DL, ed. Microbial Transformations of Low Rank Coals. Boca Raton, FL: CRC Press, pp 65–92.
Dagley S. 1975. Microbial degradation of organic compounds in the biosphere. Sci Am 63:681–689.
Davies SL, Whittenbury R. 1970. Fine structure of methane and other hydrocarbon-utilizing bacteria. J Gen Microbiol 61:227–232.
Davis CA. 1907. Peat: Essays on the Origin and Distribution in Michigan. Lansing, MI: Wynkoop Hallenbeck Crawford, State Printers.
Davis JB. 1967. Petroleum Microbiology. Amsterdam: Elsevier.
Davis JB, Yarbrough HF. 1966. Anaerobic oxidation of hydrocarbons by *Desulfovibrio desulfuricans*. Chem Geol 1:137–144.
De Bruin WP, Kotterman MJ, Posthumus MA, Schraa G, Zehnder AJ. 1992. Complete biological reductive transformation of tetrachloroethene to ethane. Appl Environ Microbiol 58:1996–2000.
Deuser WG, Degens ET, Harvey GR. 1973. Methane in Lake Kivu: New data bearing on origin. Science 181:5–54.
Didyk BM, Simoneit BRT. 1989. Hydrothermal oil of Guaymas Basin and implications for petroleum formation mechanisms. Nature (Lond) 342:65–69.
Doddema HJ, Huttenj TJ, van der Drift C, Vogels GD. 1978. ATP hydrolysis and synthesis by the membrane-bound ATP synthetase complexes of *Methanobacterium thermoautotrophicum*. J Bacteriol 136:19–23.
Doddema HJ, van der Drift C, Vogels GD, Veenhuis M. 1979. Chemiosmotic coupling in *Methanobacterium thermoautotrophicum*: Hydrogen-dependent adenosine 5′-triphosphate synthesis by subcellular particles. J Bacteriol 140:1081–1089.
Doelle HW. 1975. Bacterial Metabolism. 2nd ed. New York: Academic Press.
Dolfing J. 1990. Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth of an anaerobic bacterium, strain DCB-1. Arch Microbiol 153:264–266.
Donos JD, Frankenfeld JW. 1968. Oxidation of alkyl benzenes by a strain of *Micrococcus cerificans* growing on n-paraffins. Appl Microbiol 16:532–533.
Dugan PR. 1986. Microbiological desulfurization of coal and its increased monetary value. In: Ehrlich HL, Holmes DS, ed. Workshop on Biotechnology for the Mining, Metal-Refining and Fossil Fuel Processing Industries. Biotech Bioeng Symp 16. New York:Wiley, pp 185–203.
Dutova EN. 1962. The significance of sulfate-reducing bacteria in prospecting for oil as exemplified in the study of ground water in Central Asia. In: Kuznetsov SI, ed. Geologic Activity of Microorganisms. New York: Consultants Bureau, pp 76–78.
Dutton PL, Evans WC. 1969. The metabolism of aromatic compounds by *Rhodopseudomonas palustris*. A new reductive method of aromatic ring metabolism. Biochem J 113:525–536.

Dybas M, Konisky J. 1992. Energy transduction in the methanogen *Methanococcus voltae* is based on a sodium current. J Bacteriol 174:5575–5583.

Ehrenreich P, Behrends A, Harder J, Widdel F. 2000. Anaerobic oxidation of alkanes by newly isolated denitrifying bacteria. Arch Microbiol 173:58–64.

Ehrlich HL. 1993. Bacterial mineralization of organic carbon under anaerobic conditions. In: Bollag J-M, Stotzky G, eds. Soil Biochemistry, Vol 8. New York: Marcel Dekker, pp 219–247.

Ensley BD. 1991. Biochemical diversity of trichloroethylene metabolism. Annu Rev Microbiol 45:283–299.

Evans WC, Fuchs G. 1988. Anaerobic degradation of aromatic compounds. Annu Rev Microbiol 42:289–317.

Fenchel T, Blackburn TH. 1979. Bacteria and Mineral Cycling. London, UK: Academic Press.

Fenchel T, Finlay BJ. 1992. Production of methane and hydrogen by anaerobic ciliates containing symbiotic methanogens. Arch Microbiol 157:475–480.

Finnerty WR, Robinson M. 1986. Microbial desulfurization of fossil fuels: A review. In: Ehrlich HL, Holmes DS, eds. Workshop on Biotechnology for the Mining, Metal-Refining and Fossil Fuel Processing Industries. Biotech Bioeng Symp 16. New York: Wiley, pp 205–221.

Finnerty WR, Singer ME, King AD. 1984. Microbial processes and the recovery of heavy petroleum. In: Meyer RF, Wynne JC, Olson JC, eds. Future Heavy Crude Tar Sands. 2nd Int Conf. New York: McGraw-Hill, pp 424–429.

Finster K, Tanimoto Y, Bak F. 1992. Fermentation of methanediol and dimethylsulfide by a newly isolated methanogenic bacterium. Arch Microbiol 157:425–430.

Foght JM, Fedorak PM, Gray MR, Westlake DWS. 1990. Microbial desulfurization of petroleum. In: Ehrlich HL, Brierley CL, eds. Microbial Mineral Recovery. New York: McGraw-Hill, pp 379–407.

Foster JW. 1962. Hydrocarbons as substrates for microorganisms. Antonie v Leeuwenhoek 28:241–274.

Francis W. 1954. Coal: Its Formation and Composition. London, UK: Edward Arnold.

Fredricks KM. 1967. Products of the oxidation of n-decane by *Pseudomonas aeruginosa* and *Mycobacterium rhodochrous*. Antonie v Leeuwenhoek 33:41–48.

Fredrickson JK, Stewart DL, Campbell JA, Powell MA, McMulloch M, Pyne JW, Bean RM. 1990. Biosolubilization of low-rank coal by *Trametes versicolor* siderophore-like product and complexing agents. J Ind Microbiol 5:401–406.

Freedman DL, Gossett JM. 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl Environ Microbiol 55:2144–2151.

Gal'chenko VF, Gorlatov SN, Tokarev VG. 1986. Microbial oxidation of methane in Bering Sea sediments. Mikrobiologiya 55:669–673 (Engl transl, pp 526–530).

Giani D, Giani L, Cohen Y, Krumbein WE. 1984. Methanogenesis in the hypersaline Solar Lake (Sinai). FEMS Microbiol Lett 25:219–224.

Gold T, Gordon BE, Streett W, Bilson E, Panaik P. 1986. Experimental study of the reaction of methane with petroleum hydrocarbons in geological conditions. Geochim Cosmochim Acta 50:2411–2418.

Gorlatov SN, Balyaev SS. 1984. The aerobic microflora of an oil field and its ability to destroy petroleum. Mikrobiologiya 53:843–849 (Engl transl, pp 701–706).
Gorlatov SN, Gal'chenko VF, Tokarev VG. 1986. Microbiological methane formation in deposits of the Bering Sea. Mikrobiologiya 55:490–495 (Engl transl, pp 380–385).
Gottschalk G. 1986. Bacterial Metabolism. 2nd ed. New York: Springer-Verlag.
Gottschalk G, Blaut M. 1990. Generation of proton and sodium motive forces in methanogenic bacteria. Biochim Biophys Acta 1018:263–266.
Grbic-Galic D. 1990. Methanogenic transformation of aromatic hydrocarbons and phenols in groundwater aquifers. Geomicrobiol J 8:167–200.
Grossman MJ, Lee MK, Prince RC, Minak-Bernero V, George GN, Pickering IJ. 2001. Deep desulfurization of extensively hydrodesulfurized middle distillate oil by *Rhodococcus* sp. strain ECRD-1. Appl Environ Microbiol 67:1949–1952.
Hansen LB, Finster K, Fossing H, Iversen N. 1998. Anaerobic methane oxidation in sulfate depleted sediments: Effects of sulfate and molybdate additions. Aquat Microb Ecol 14:195–204.
Henrichs SM, Reeburgh WS. 1987. Anaerobic mineralization of organic matter: Rates and the role of anaerobic processes in the oceanic carbon economy. Geomicrobiol J 5:191–237.
Higgins IJ, Best DJ, Hammond RC, Scott D. 1981. Methane-oxidizing microorganisms. Microbiol Rev 45:556–590.
Higson FK. 1992. Microbial degradation of biphenyl and its derivatives. Adv Appl Microbiol 37:135–164.
Hoehler TM, Alperin MJ, Albert DB, Martens CS. 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen–sulfate reducer consortium. Global Biogeochem Cycles 8:451–463.
Hoehler TM, Alperin MJ, Albert DB, Martens CS. 1998. Thermodynamic control on hydrogen concentrations in anoxic sediments. Geochim Cosmochim Acta 62:1745–1756.
Horita J, Berndt ME. 1999. Abiogenic methane formation and isotope fractionation under hydrothermal conditions. Science 285:1055–1057.
Horvath RS. 1972. Microbial catabolism and the degradation of organic compounds in nature. Bacteriol Rev 36:146–155.
Huber H, Thomm M, König G, Thies G, Stetter KO. 1982. *Methanococcus thermolithotrophicus*, a novel thermophilic lithotrophic methanogen. Arch Microbiol 132:47–50.
Hunt JM. 1984. Generation and migration of light hydrocarbons. Science 226:1265–1270.
Hunt JM, Whelan JK, Huc AY. 1980. Genesis of petroleum hydrocarbons in marine sediments. Science 209:403–404.
Huser BA, Wuhrmann K, Zehnder AJB. 1982. *Methanothrix soehngii* gen. nov. spec. nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. Arch Microbiol 132:1–9.
Imhoff-Stuckle D, Pfennig N. 1983. Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. Arch Microbiol 136:194–198.
Ivanov MV. 1967. The development of geological microbiology in the U.S.S.R. Mikrobiologiya 36:849–859 (Engl transl, pp 715–722).

Ivanov MV, Nesterov AI, Nasaraev GB, Gal'chenko VF, Nazarenko AV. 1978. Distribution and geochemical activity of methanotrophic bacteria in coal mine waters. Mikrobiologiya 47:489–494 (Engl. transl. pp 396–401).

Iversen N, Jørgensen BB. 1985. Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). Limnol Oceanogr 30:944–955.

Izumi Y, Ohshiro T, Ogino H, Hine Y, Shimao M. 1994. Selective desulfurization of dibenzothiophene by *Rhodococcus erythropolis* D-1. Appl Environ Microbiol 60:223–226.

Jain MK, Zeikus JG. 1989. Bioconversion of gelatin to methane by a coculture of *Clostridium collagenovorans* and *Methanosarcina barkeri*. Appl Environ Microbiol 55:366–371.

Jakobsen P, Patrick WH Jr, Williams BG. 1981. Sulfide and methane formation in soils and sediments. Soil Sci 132:279–287.

Jobson AM, Cook FD, Westlake DWS. 1979. Interaction of aerobic and anaerobic bacteria in petroleum biodegradation. Chem Geol 24:355–365.

Johnson MJ. 1964. Utilization of hydrocarbons by microorganisms. Chem Ind 36:1532–1537.

Jones JG, Simon BM, Gardener S. 1982. Factors affecting methanogenesis and associated anaerobic processes in the sediments of a stratified eutrophic lake. J Gen Microbiol 128:1–11.

Jones RD, Morita RY. 1983. Methane oxidation by *Nitrosococcus oceanus* and *Nitrosomonas europaea*. Appl Environ Microbiol 45:401–410.

Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS. 1983a. *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. Arch Microbiol 136:254–261.

Jones WJ, Paynter MJB, Gupta R. 1983b. Characterization of *Methanococcus maripaludis* sp. nov., a new methanogen from salt marsh sediment. Arch Microbiol 135:91–97.

Jones WJ, Nagle DP Jr, Whitman WB. 1987. Methanogens and the diversity of archaebacteria. Microbiol Rev 51:135–177.

Klein DA, Davis JA, Casida LE. 1968. Oxidation of n-alkanes to ketones by an *Arthrobacter* species. Antonie v Leeuwenhoek 34:495–503.

Koburger JA. 1964. Microbiology of coal: Growth of bacteria in plain and oxidized coal slurries. 39th Annu Session of W Virginia Acad Sci Proc. W VA Acad Sci 36:26–30.

Kristjansson JK, Schönheit P, Thauer RK. 1982. Different K_s values for hydrogen of methanogenic and sulfate-reducing bacteria: An explanation for the apparent inhibition of methanogenesis by sulfate. Arch Microbiol 131:278–282.

Kulm LD, Suess E, Moore JC, Carson B, Lewis BT, Ritger SD, Kadko DC, Thornburg TM, Embley RW, Rugh WD, Massoth GJ, Langseth MG, Cochrane GR, Scamman RL. 1986. Oregon subduction zone: Venting, fauna, and carbonates. Science 231:561–566.

Kuznetsov SI, Ivanov MV, Lyalikova NN. 1963. Introduction to Geological Microbiology. New York: McGraw-Hill.

Kuznetsova VA, Gorlenko VM. 1965. The growth of hydrocarbon-oxidizing bacteria under anaerobic conditions. Prikl Biokhim Mikrobiol 1:623–626.

Kvasnikov EI, Lipshits VV, Zubova NV. 1973. Facultative anaerobic bacteria of producing petroleum wells. Mikrobiologiya 42:925–930 (Engl transl, pp 823–827).

Larsson L, Olsson G, Holst O, Karlsson HT. 1990. Pyrite oxidation by thermophilic archaebacteria. Appl Environ Microbiol 56:697–701.
Leadbetter ER, Foster JW. 1959. Oxidation products formed from gaseous alkanes by the bacterium *Pseudomonas methanica*. Arch Biochem Biophys 82:491–492.
Lovley DR. 1987. Organic matter mineralization with the reduction of ferric iron: A review. Geomicrobiol J 5:375–399.
Lovley DR. 1993. Dissimilatory metal reduction. Annu Rev Microbiol 47:263–290.
Lovley DR, Phillips EJP. 1988. Novel mode of microbial energy metabolism: Organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl Environ Microbiol 54:1472–1480.
MacDonald IR, Reilly JF, Guinasso NL Jr, Brooks JM, Carney RS, Bryant WA, Bright TJ. 1990. Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico. Science 248:1096–1099.
MacLeod FA, Guiot SR, Costerton JW. 1990. Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. Appl Environ Microbiol 56:1598–1607.
Madigan MT, Gest H. 1978. Growth of a photosynthetic bacterium anaerobically in darkness, supported by "oxidant-dependent" sugar fermentation. Arch Microbiol 117:119–122.
Mah RA, Smith MR, Baresi L. 1978. Studies on an acetate-fermenting strain of Methanosarcina. Appl Environ Microbiol 35:1174–1184.
Mango FD. 1992. Transition metal catalysis in the generation of petroleum and natural gas. Geochim Cosmochim Acta 56:3851–3854.
Martens CS. 1976. Control of methane sediment-water bubble transport by macroinfaunal irrigation in Cape Lookout Bight, North Carolina. Science 192:998–1000.
Martens CS, Berner RA. 1974. Methane production in the interstitial waters of sulfate-depleted marine sediments. Science 185:1167–1169.
Martens CS, Klump JV. 1984. Biogeochemical cycling in an organic-rich coastal marine basin. 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. Geochim Cosmochim Acta 48:1987–2004.
Maymó-Gatell X, Anguish T, Zinder SH. 1999. Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "*Dehalococcoides ethenogenes*" 195. Appl Environ Microbiol 65:3108–3113.
McInerney MJ, Westlake DWS. 1990. Microbially enhanced oil recovery. In: Ehrlich HL, Brierley CL, eds. Microbial Mineral Recovery. New York: McGraw-Hill, pp 409–445.
McInerney MJ, Bryant MP, Pfennig N. 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch Microbiol 122:129–135.
McKenna EJ, Kallio RE. 1964. Hydrocarbon structure: Its effect on bacterial utilization of alkanes. In: Heukelakian H, Dondero N, eds. Principles and Applications in Aquatic Microbiology. New York: Wiley, pp 1–14.
Merrettig U, Wlotzta P, Onken U. 1989. The removal of pyritic sulfur from coal by *Leptospirillum*-like bacteria. Appl Microbiol Biotechnol 31:626–628.
Meyer WC, Yen TF. 1976. Enhanced dissolution of oil shale by bioleaching with thiobacilli. Appl Environ Microbiol 32:610–616.
Mohn WW, Tiedje JM. 1990. Strain DCB-1 conserves energy for growth from reductive dechlorination couple to formate oxidation. Arch Microbiol 153:267–271.

Mohn WW, Tiedje JM. 1991. Evidence for chemiosmotic coupling of reductive dechlorination and ATP synthesis in *Desulfomonile tiedjei*. Arch Microbiol 157:1–6.

Mormile MR, Atlas RM. 1988. Mineralization of dibenzothiophene biodegradation products 3-hydroxy-2-formyl benzothiophene and dibenzothiophene sulfone. Appl Environ Microbiol 54:3183–3184.

Mountford DO. 1978. Evidence for ATP synthesis driven by a proton gradient in *Methanosarcina barkeri*. Biochem Biophys Res Commun 85:1346–1351.

Müller V, Blaut M, Gottschalk B. 1993. Bioenergetics of methanogenesis. In: Ferry JG, ed. Methanogenesis. New York: Chapman and Hall, pp 360–406.

Myers CR, Nealson KH. 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science 240:1319–1321.

Nazina TN, Rozanova EP. 1978. Thermophilic sulfate-reducing bacteria from oil strata. Mikrobiologiya 47:142–148 (Engl transl, pp 113–118).

Nazina TN, Rozanova EP. 1980. Ecological conditions for the distribution of methane producing bacteria in oil-containing strata of Apsheron. Mikrobiologiya 49:123–129 (Engl transl, pp 104–109).

Nazina TN, Rozanova EP, Kuznetsov SI. 1985. Microbial oil transformation processes accompanied by methane and hydrogen-sulfide formation. Geomicrobiol J 4:103–130.

Nette IT, Grechushkina NN, Rabotnova IL. 1965. Growth of certain mycobacteria in petroleum and petroleum products. Prikl Biokhim Mikrobiol 1:167–174.

Niewöhner C, Hensen C, Kasten S, Zabel M, Schulz HD. 1998. Deep sulfate reduction completely mediated by anaerobic methane oxidation in sediments of the upwelling area off Namibia. Geochim Cosmochim Acta 62:455–464.

North FK. 1985. Petroleum Geology. Boston, MA: Allen and Unwin.

Olson ES, Stanley DC, Gallagher JR. 1993. Characterization of intermediates in the microbial desulfurization of dibenzothiophene. Energy Fuels 7:159–164.

Omori T, Monna L, Saiki Y, Kodama T. 1992. Desulfurization of dibenzothiophene by *Corynebacterium* sp. strain SY1. Appl Environ Microbiol 58:911–915.

O'Neill JG, Wilkinson JF. 1977. Oxidation of ammonia by methane-oxidizing bacteria and the effects of ammonia on methane oxidation. J Gen Microbiol 100:407–412.

Oremland RS, Taylor BF. 1978. Sulfate reduction and methanogenesis in marine sediments. Geochim Cosmochim Acta 42:209–214.

Oremland RS, Marsh LM, Polcin S. 1982. Methane production and simultaneous sulfate reduction in anoxic, salt marsh sediments. Nature (Lond) 296:143–145.

Orphan VJ, Hinrichs K-U, Ussler W III, Paull CK, Taylor LT, Sylva SP, Hayes JM, DeLong EF. 2001. Comparative analysis of methane-oxidizing Archaea and sulfate-reducing Bacteria in anoxic marine sediments. Appl Environ Microbiol 67:1922–1934.

Orr FM Jr, Taber JJ. 1984. Use of carbon dioxide in enhanced oil recovery. Science 224:563–569.

Ourisson G, Albrecht P, Rohmer M. 1984. Microbial origin of fossil fuels. Sci Am 251:44–51.

Pancost RD, Damsté JSS, de Lint S, van der Maarel MJEC, Gottschal JC, Medinaut Shipboard Scientific Party. 2000. Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean sediments by a consortium of methanogenic Archaea and Bacteria. Appl Environ Microbiol 66:1126–1132.

Panganiban A, Hanson RS. 1976. Isolation of a bacterium that oxidizes methane in the absence of oxygen. Abstr, Annu Meet Am Soc Microbiol, 159:121.
Panganiban AT Jr, Patt TE, Hart W, Hanson RS. 1979. Oxidation of methane in the absence of oxygen in lake water samples. Appl Environ Microbiol 37:303–309.
Paszczynski A, Huynh V-B, Crawford R. 1986. Comparison of lignase-1 and peroxidase-M2 from the white-rot fungus *Phanerochaete chrysosporium*. Arch Biochem Biophys 244:750–785.
Patel GB. 1984. Characterization and nutritional properties of *Methanothrix concilii* sp. nov., an aceticlastic methanogen. Can J Microbiol 30:1383–1396.
Phillips WE Jr, Perry JJ. 1974. Metabolism of n-butane and 2-butanone by *Mycobacterium vaccae*. J Bacteriol 120:987–989.
Pirnik MP, Atlas RM, Bartha R. 1974. Hydrocarbon metabolism by *Brevibacterium erythrogenes*: Normal and branched alkanes. J Bacteriol 119:868–878.
Pooley FD, Atkins AS. 1983. Desulfurization of coal using bacteria by both dump and process plant techniques. In: Rossi G, Torma AE, eds. Recent Progress in Biohydrometallurgy. Iglesias, Italy: Assoc Minerar Sarda, pp 511–526.
Pyne JW Jr, Stewart DL, Fredrickson J, Wilson BW. 1987. Solubilization of leonardite by an extracellular fraction from *Coriolus versicolor*. Appl Environ Microbiol 53:2844–2848.
Quayle JR, Ferenci T. 1978. Evolutionary aspects of autotrophy. Microbiol Rev 42:251–273.
Radway J, Tuttle JH, Fendinger NJ, Means JC. 1987. Microbially mediated leaching of low-sulfur coal in experimental coal columns. Appl Environ Microbiol 53:1056–1063.
Radway J, Tuttle JH, Fendinger NJ. 1989. Influence of coal source and treatment upon indigenous microbial communities. J Ind Microbiol 4:195–208.
Raiders RA, Knapp RM, McInerney JM. 1989. Microbial selective plugging and enhanced oil recovery. J Ind Microbiol 4:215–230.
Raymond RL, Jamison VW, Hudson JO. 1967. Microbial hydrocarbon cooxidation. I. Oxidation of mono- and dicyclic hydrocarbons by soil isolates of the genus *Nocardia*. Appl Microbiol 15:857–865.
Reeburgh WS. 1976. Methane consumption in Cariaco Trench waters and sediments. Earth Planet Sci Lett 28:337–344.
Reeburgh WS. 1980. Anaerobic methane oxidation: Rate depth distribution in Skan Bay sediments. Earth Planet Sci Lett 47:345–352.
Reineke W, Knackmuss H-J. 1988. Microbial degradation of haloaromatics. Annu Rev Microbiol 42:263–287.
Rivard CJ, Smith PH. 1982. Isolation and characterization of a thermophilic marine methanogenic bacterium, *Methanogenium thermophilicum* sp. nov. Int J Syst Bacteriol 32:430–436.
Robertson R. 1966. The origins of petroleum. Nature (Lond) 212:1291–1295.
Robinson JA, Tiedje JM. 1984. Competition between sulfate-reducing and methanogenic bacteria for H_2 under resting and growing conditions. Arch Microbiol 137:26–32.
Roffey R, Norqvist A. 1991. Biodegradation of bitumen used for nuclear waste disposal. Experientia 47:539–542.
Rogoff MH, Wander I, Anderson RB. 1962. Microbiology of Coal. Info Circ 8057. Washington, DC: Bur Mines, US Dept of the Interior.

Roitsch T, Stolp H. 1985. Distribution of dissimilatory enzymes in methane and methanol oxidizing bacteria. Arch Microbiol 143:233–236.

Romanovskaya VA, Lyudvichenko ES, Kryshtab TP, Zhukov VG, Sokolov IG, Malashenko YuR. 1980. Role of exogenous carbon dioxide in metabolism of methane-oxidizing bacteria. Mikrobiologiya 49:687–693 (Engl transl, pp 566–571).

Romesser JA, Wolfe RS, Mayer F, Spiess E, Walther-Mauruschat A. 1979. *Methanogenium*, a new genus of marine methanogenic bacteria, and characterization of *Methanogenium cariaci* sp. nov. and *Methanogenium marisnigri* sp. nov. Arch Microbiol 121:147–153.

Rozanova EP. 1971. Morphology and certain physiological properties of purple bacteria from oil-bearing strata. Mikrobiologiya 40:152–157 (Engl transl, pp 134–138).

Rozanova EP. 1978. Sulfate reduction and water-soluble organic substances in a flooded oil reservoir. Mikrobiologiya 47:495–500 (Engl transl, pp 401–405).

Rubinshtein LM, Oborin AA. 1986. Microbial methane production in stratal waters of oilfields in the Perm area of the Cis-Ural region. Mikrobiologiya 55:674–678 (Engl transl, pp 530–534).

Rubinstein I, Strausz OP, Spyckerelle C, Crawford RJ, Westlake DW. 1977. The origin of oil sand bitumens of Alberta: A chemical and a microbiological study. Geochim Cosmochim Acta 41:1341–1353.

Sansone FJ, Martens CS. 1981. Methane production from acetate and associated methane fluxes from anoxic coastal sediments. Science 211:707–709.

Schönheit P, Kristjansson JK, Thauer RK. 1982. Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. Arch Microbiol 132:285–288.

Sharak Genthner BR, Price WA II, Pritchard PH. 1989. Anaerobic degradation of chloroaromatic compounds in aquatic sediments under a variety of enrichment conditions. Appl Environ Microbiol 55:1466–1477.

Shelton DR, Tiedje JM. 1984. Isolation and characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl Environ Microbiol 48:840–848.

Sieburth JMcN, Johnson PW, Eberhardt MA, Sieracki ME, Lidstrom ME, Laux D. 1987. The first methane-oxidizing bacterium from the upper mixing layer of the deep ocean: *Methylomonas pelagica* sp. nov. Curr Microbiol 14:285–293.

Simakova TL, Kolesnik ZA, Strigaleva NV, et al. 1968. Transformation of high-paraffinaceous oil by microorganisms under anaerobic and aerobic conditions. Mikrobiologiya 37:233–238 (Engl transl, pp 194–198).

Simoneit BRT, Lonsdale PF. 1982. Hydrothermal petroleum in mineralized mounds at the seabed of Guaymas Basin. Nature (Lond) 295:198–202.

Simpson PG, Whitman WB. 1993. Anabolic pathways in methanogens. In: Ferry JG, ed. Methanogenesis. New York: Chapman and Hall, pp 445–472.

Skyring GW. 1987. Sulfate reduction in coastal ecosystems. Geomicrobiol J 5:295–374.

Smith MR, Mah RA. 1978. Growth and methane genesis by *Methanosarcina* strain 227 on acetate and methanol. Appl Environ Microbiol 36:870–879.

Sokolov IG. 1986. Coupling of the process of electron transport to methane monooxygenase with the translocation of protons in methane-oxidizing bacteria. Mikrobiologiya 55:715–722 (Engl transl., pp 559–565).

Sokolov IG, Malashenko YuR, Romanovskaya VA. 1981. Electron transport chain of thermophilic methane-oxidizing culture *Methylococcus thermophilus*. Mikrobiologiya 50:13–20 (Engl transl, pp 7–13).

Sørensen J. 1987. Nitrate reduction in marine sediment: Pathways and interactions with iron and sulfur cycling. Geomicrobiology 5:401–421.

Sprott GD, Bird SE, MacDonald IJ. 1985. Proton motive force as a function of the pH at which *Methanobacterium bryantii* is grown. Can J Microbiol 31:1031–1034.

Stanier RY, Ingraham JL, Wheelis ML, Painter PR. 1986. The Microbial World, 5th ed. Englewood Cliffs, NJ: Prentice-Hall.

Stetter KO, Gaag G. 1983. Reduction of molecular sulfur by methanogenic bacteria. Nature (Lond) 305:309–311.

Stetter KO, Huber R, Boechl E, Kurr M, Eden RD, Fielder M, Cash H, Vance I. 1993. Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. Nature (Lond) 365:743–745.

Stevenson IL. 1967. Utilization of aromatic hydrocarbons by *Arthrobacter* spp. Can J Microbiol 13:205–211.

Stewart DL, Thomas BL, Bean RM, Fredrickson JK. 1990. Colonization and degradation of bituminous and lignite coals by fungi. J Ind Microbiol 6:53–59.

Stewart JE, Kallio RE, Stevenson DP, Jones AC, Schissler DO. 1959. Bacterial hydrocarbon oxidation. I. Oxidation of n-hexadecane by a gram-negative coccus. J Bacteriol 78:441–448.

Stoner DL, Wey JW, Barrett KB, Jolley JG, Wright RB, Dugan PR. 1990. Modification of water-soluble coal-derived products by dibenzothiophene-degrading microorganisms. Appl Environ Microbiol 56:2667–2676.

Szewzyk R, Pfennig N. 1987. Complete oxidation of catechol by the strictly anaerobic sulfate reducing *Desulfobacterium catecholicum* sp. nov. Arch Microbiol 147:163–168.

Tanner RS, Udegbunam EO, McInerney MJ, Knapp RM. 1991. Microbially enhanced oil recovery from carbonate reservoirs. J Ind Microbiol 9:169–195.

Teh JS, Lee KH. 1973. Utilization of n-alkanes by *Cladosporium resinae*. Appl Microbiol 25:454–457.

Thomsen TR, Finster K, Ramsing NB. 2001. Biogeochemical and molecular signatures of anaerobic methane oxidation in a marine sediment. Appl Environ Microbiol 67:1646–1656.

Townsley CC, Atkins AS, Davis AJ. 1987. Suppression of pyritic sulfur during flotation tests using the bacterium *Thiobacillus ferrooxidans*. Biotech Bioeng 30:1–8.

Van Afferden M, Schacht S, Klein J, Tüper HG. 1990. Degradation of dibenzothiophene by *Brevibacterium* sp. DO. Arch Microbiol 153:324–328.

Van Bruggen JJA, Zwart KB, van Assema RM, Stumm CK, Vogels GD. 1984. *Methanobacterium formicum*, an endosymbiont of the anaerobic ciliate *Metopus striatus* McMurrich. Arch Microbiol 139:1–7.

Van der Linden AC, Thijsse GJE. 1965. The mechanism of microbial oxidations of petroleum hydrocarbons. Adv Enzymol 27:469–546.

Vogels GD. 1979. The global cycle of methane. Antonie v Leeuwenhoek 45:347–352.

Vogels GD, Visser CM. 1983. Interconnections of methanogenic and acetogenic pathways. FEMS Microbiol Lett 20:291–297.

Volesky B, Zajic JE. 1970. Ethane and natural gas oxidation by fungi. Dev Ind Microbiol 11:184–195.

Walker JD, Cooney JJ. 1973. Pathway of n-alkane oxidation in *Cladosporium resinae*. J Bacteriol 115:635–639.

Ward BB, Kilpatrick KA, Novelli PC, Scranton MI. 1987. Methane oxidation and methane fluxes in the ocean surface layer and deep anoxic layers. Nature (Lond) 327:226–229.

Ward DM, Brock TD. 1978. Anaerobic metabolism of hexadecane in sediments. Geomicrobiol J 1:1–9.

Westlake DWS, Belicek W, Jobson A, Cook FD. 1976. Microbial utilization of raw and hydrogenated shale oils. Can J Microbiol 22:221–227.

Whittenbury R, Phillips KC, Wilkinson JF. 1970. Enrichment isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 61:205–218.

Widdel F. 1986. Growth of methanogenic bacteria in pure culture with 2-propanol and other alcohols as hydrogen donors. Appl Environ Microbiol 51:1056–1062.

Wildgruber G, Thomm M, König H, Ober K, Ricchiuto T, Stetter KO. 1982. *Methanoplanus limicola*, a plate-shaped methanogen representing a novel family, the Methanoplanaceae. Arch Microbiol 132:31–36.

Winter J, Wolfe RS. 1979. Complete degradation of carbohydrate to carbon dioxide and methane by syntrophic cultures of *Acetobacter woodii* and *Methanosarcina barkeri*. Arch Microbiol 121:97–102.

Winter JU, Wolfe RS. 1980. Methane formation from fructose by syntrophic associations of *Acetobacterium woodii* and different strains of methanogens. Arch Microbiol 124: 73–79.

Wolf M, Bachofen R. 1991. Microbial degradation of bitumen. Experientia 47:542–548.

Wolin MJ. 1981. Fermentation in the rumen and human large intestine. Science 213:1463–1468.

Wolin MJ, Miller TL. 1987. Bioconversion of organic carbon to CH_4 and CO_2. Geomicrobiol J 5:239–259.

Worakit S, Boone DR, Mah RA, Abdel-Samie M-E, El-Halwagi MM. 1986. *Methanobacterium alcaliphilum* sp. nov., an H_2-utilizing methanogen that grows at high pH values. Int J Syst Bacteriol 36:380–382.

Yang KY-L, Lapado J, Whitman WB. 1992. Pyruvate oxidation by *Methanococcus* spp. Arch Microbiol 158:271–275.

Yarbrough HF, Coty VF. 1983. Microbially enhanced oil recovery from the Upper Cretaceous Nacatoch formation, Union County, Arkansas. In: Donaldson EC, Clark JB, eds. Proc Int Conf Microb Enhancement Oil Recovery (Conf 8205140). Springfield, VA: NTIS, pp 149–153.

Yavitt JB, Lang GE. 1990. Methane production in contrasting wetland sites: Response to organic-chemical components of peat and to sulfate reduction. Geomicrobiol J 8:27–46.

Yen HC, Marrs B. 1977. Growth of *Rhodopseudomonas capsulata* under anaerobic dark conditions with dimethyl sulfoxide. Arch Biochem Biophys 181:411–418.

Young LY, Frazer AC. 1987. The fate of lignin and lignin-derived compounds in anaerobic environments. Geomicrobiol J 5:261–293.

Zabel HP, König H, Winter J. 1984. Isolation and characterization of a new coccoid methanogen, *Methanogenium tatii* spec. nov. from a solfataric field on Mount Tatio. Arch Microbiol 137:308–315.

Zehnder AJB, Brock TD. 1979. Methane formation and methane oxidation by methanogenic bacteria. J Bacteriol 137:420–432.

Zehnder AJB, Huser BA, Brock TD, Wuhrmann K. 1980. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch Microbiol 124:1–11.

Zeikus JG. 1977. The biology of methanogenic bacteria. Bacteriol Rev 41:514–541.

Zeikus JG, Kerby R, Krzycki JA. 1985. Single-carbon chemistry of acetogenic and methanogenic bacteria. Science 127:1167–1173.

Zellner G, Bleicher K, Braun E, Kneifel H, Tindall BJ, Conway de Marcario E, Winter J. 1989. Characterization of a new mesophilic, secondary alcohol-utilizing methanogen, *Methanobacterium palustre* spec. nov. from a peat bog. Arch Microbiol 151:1–9.

Zellner G, Sleytr UB, Messner P, Kneifel H, Winter J. 1990. *Methanogenium liminatans* spec. nov., a new coccoid, mesophilic methanogen able to oxidize secondary alcohols. Arch Microbiol 153:287–293.

Zimmerman PR, Greenberg JP, Windiga SO, Crutzen PJ. 1982. Termites: A potentially large source of atmospheric methane, carbon dioxide and molecular hydrogen. Science 218:563–565.

Zinder SH. 1993. Physiological ecology of methanogens. In: Ferry JG, ed. Methanogenesis. New York: Chapman and Hall, pp 128–206.

Zinder SH, Koch M. 1984. Non-aceticlastic methanogenesis from acetate: Acetate oxidation by a thermophilic syntrophic coculture. Arch Microbiol 138:263–272.

ZoBell CE. 1952. Part played by bacteria in petroleum formation. J Sedimen Petrol 22:42–49.

ZoBell CE. 1963. The origin of oil. Int Sci Technol August: 42–48.

Zvyagintsev DG, Zenova GM, Shirokykh IG. 1993. Distribution of actinomycetes with the vertical structure of peat bog ecosystems. Mikrobiologiya 62:548–555 (Engl transl., pp 339–342).

Glossary

Acetogen A bacterial culture that forms acetic acid as the sole product in the reduction of CO_2 with hydrogen, or in the fermentation of a sugar. Also called homoacetogen.
Acidophilic bacteria Bacteria that need an acid environment in which to grow.
Actinomycete Mycelium-forming bacterium; found in soil and aquatic environments.
Adenosine 5′-diphosphate (ADP) A phosphate anhydride that serves as acceptor of high-energy phosphate in metabolic energy conservation, or it may be a product in enzymatic phosphorylation with ATP. It contains one high-energy phosphate bond.
Adenosine 5′-triphosphate (ATP) A phosphate anhydride that conserves metabolic energy. It contains two high-energy phosphate bonds.
Adipocere A waxy substance from dead organic matter; a soap.
Adventitious organisms Organisms introduced naturally from an adjacent habitat; they may or may not be able to grow or survive in the new habitat.
Aerobe An organism that lives in air and uses oxygen as terminal electron acceptor in its respiratory process.
Aerobic heterotroph An organism that uses organic substances as carbon and energy sources in air (oxygen-respiring).
Agar (*also* **agar agar)** A polysaccharide heteropolymer derived from the walls of certain red algae and used for gelling bacteriological culture media.
Agar shake culture A bacterial culture method in which the bacterial inoculum is completely mixed in an agar medium in a test tube.

Allochthonous Introduced from another place.
Aluminosilicate A mineral containing a combination of aluminum and silicate.
Amictic lake A lake that never turns over.
Ammonification A biochemical process that releases amino nitrogen as ammonia from organic compounds such as proteins and amino acids.
Amorphous Noncrystalline.
AMP Adenylic acid; adenosine monophosphate.
Amphibole A ferromagnesian mineral with two infinite chains of silica tetrahedra linked to each other; the double chains are cross-linked by Ca, Mg, and Fe.
Amphoteric Having both acidic and basic properties.
Anabolism That part of metabolism that deals with synthesis and polymerization of biomolecules in an energy-consuming process.
Anaerobe An organism that grows in the absence of oxygen; it may be oxygen-tolerant or -intolerant.
Anaerobic heterotrophy A form of nutrition using organic energy and carbon sources in the absence of oxygen.
Anaerobic respiration A respiratory process in which nitrate, sulfate, sulfur, carbon dioxide, Fe(III), Mn(IV), or some other externally supplied, reducible inorganic or organic compounds substitute for oxygen as terminal electron acceptor.
Anhydrite A calcium sulfate mineral, $CaSO_4$.
Anodic surface A surface exhibiting a net positive charge.
Anticyclones In oceanography, a small closed-current system of water derived from a surface current; it has a warm core surrounded by colder water, spinning in a clockwise rotation (*see also* Rings; Meddies).
APS Adenosine phosphosulfate; adenosine sulfatophosphate.
Aragonite A calcium carbonate mineral forming slender pointed crystals.
Archaea (formerly **Archaebacteria, Archaeobacteria**) A domain of the Prokaryotes that includes methanogens, *Sulfolobus*, *Acidianus*, *Halobacterium*, and *Thermoplasma*, among other genera, that have a unique cell envelope and plasma membrane structure and a unique type of ribosomal RNA that distinguishes it from the domain Bacteria.
Archaeon A member of the Archaea.
Aridisol A mature desert soil.
Arroyo A dried-up riverbed in a desert region through which water flows after a rainstorm; a wash.
Arsenopyrite An iron-arsenic sulfide mineral, FeAsS.
Arsine AsH_3.
Ascomycetes Fungi that deposit their sexual spores in sacs (asci), e.g., *Neurospora*.
Asparagine The amide of aspartic acid, a dicarboxylic amino acid.
Assimilation Uptake and incorporation of nutrients by cells.

Asthenosphere Upper portion of the Earth's mantle, which is thought to have a plastic consistency and upon which the crustal plates float.
Atmosphere The gaseous envelope around the Earth.
ATP Adenosine 5′-triphosphate.
Augite A pyroxene type of mineral.
Authigenic Formed de novo from dissolved species in the case of minerals.
Autochthonous Generated in place; indigenous.
Autotroph An organism capable of growth exclusively at the expense of inorganic nutrients.
Bacteria A domain of the prokaryotes; formerly called Eubacteria.
Bacterioneuston The bacterial population located in a thin film at the air/water interface in a natural body of water.
Bacterioplankton Unattached bacterial forms in an aqueous environment.
Bacterium A prokaryotic single- or multicelled organism. Single cells may appear as rods, spheres, spirals, or other shapes.
Baltica A former continent encompassing Russia west of the Urals, Scandinavia, Poland, and northern Germany.
Banded Iron Formation (BIF) A sedimentary deposit featuring alternating iron oxide–rich (Fe_2O_3 or Fe_3O_4) layers and iron oxide–poor cherty layers; thought to have originated in the Precambrian at the time of transition from a nonoxidizing to an oxidizing atmosphere due to the buildup of O_2 in it.
Barium psilomelane A complex manganese(IV) oxide.
Barophile An organism capable of growth at elevated hydrostatic pressure.
Barren solution Pregnant solution from an ore leaching operation after its valuable metals have been removed.
Basaltic rock Rock of volcanic origin showing very fine crystallization due to rapid cooling. Basalt is rich in pyroxenes and feldspars.
Basidiomycetes Fungi that form sexual spores on basidia (club-shaped cells) and feature septate mycelia (e.g., mushrooms).
Benthic Located at the bottom of a body of water.
Betaine $HOOCCH_2N^+(CH_3)_3$.
Binary fission Cell division in which one cell divides into two cells of approximately equal size.
Bioherm A large mineral aggregate of biological origin; a microbialite.
Bioleaching A process whereby microbes extract metal values from ore by solubilizing them through oxidation, reduction, or complexation.
Biosphere The portion of the Earth inhabited by living organisms.
Birnessite A manganese(IV) oxide mineral, δMnO_2.
Bisulfite HSO_3^-.
Calcareous ooze A sediment having calcareous structures from foraminifera, coccolithophores, or other $CaCO_3$-depositing organisms as major constituents.
Calcite A calcium carbonate mineral with a rhombohedral structure.

Capillary culture method The use of a glass capillary with optically flat sides inserted into soil or sediment for culturing microbes from these sources in situ. Developing microbes in the capillaries may be observed directly under a microscope after withdrawal of the capillaries from the soil or sediment.
Catabolism That part of metabolism which involves degradation of nutrients and energy conservation from their oxidation.
Catalase An enzyme capable of catalyzing the reaction $H_2O_2 \rightarrow H_2O + 0.5O_2$; it can also catalyze the reduction of H_2O_2 with an organic hydrogen donor or inorganic electron donor.
Cathodic surface A surface exhibiting a net negative charge.
Celestite A strontium sulfate mineral, $SrSO_4$.
Cellulolytic Capable of enzymatic hydrolysis of cellulose.
Centric geometry Cylindrical, in reference to diatoms.
Chalcopyrite A copper-iron sulfide, $CuFeS_2$.
Chasmolithic Living inside preformed pores, fissures, or cavities in rock.
Chemocline A chemical gradient zone in a water column that separates a more dilute and less dense phase from a more concentrated phase.
Chemolithotroph An autotroph that derives energy from the oxidation of inorganic matter.
Chemostat A culture system permitting microbial growth under steady-state conditions.
Chlorophyll A light-harvesting and energy-transducing type of pigment of photosynthetic organisms.
Chloroplasts Photosynthetic organelles in eukaryotic cells.
Choline $HOCH_2CH_2N^+(CH_3)_3$.
Coccolithophore A chrysophyte alga whose surface is covered with $CaCO_3$ platelets (coccoliths).
Colony counting A method of enumerating viable, culturable microbes by counting colonies (visible aggregates) formed by them on and/or in agar medium in a Petri dish or test tube.
Conjugation Unidirectional transfer of genetic information between prokaryotic cells that requires cell-to-cell contact.
Connate waters Saline water trapped in rock strata in the geologic past, usually having undergone chemical alteration through reaction with the enclosing rock.
Consortium An association of two or more different microbes that exhibit a metabolic interdependence.
Constitutive enzyme An enzyme that is always present in an active form in a cell, whether needed or not.
Contaminant An organism accidentaly introduced during experimental manipulation of a habitat.

Continental drift Migration of continents on the Earth's surface as a result of crustal plate motions.
Continental margin The edge of a continent.
Continental rise Gently sloping seafloor at the base of the continental slope.
Continental shelf Gently sloping seafloor between the shore and the continental slope.
Continental slope Steeply sloping seafloor at the outer edge of the continental shelf.
Convergence The confluence of two water masses.
Co-oxidation Simultaneous microbial oxidation of two compounds, which may be quite unrelated, only one of which supports growth.
Copiotroph A bacterium that requires a nutrient-rich environment to grow.
Coriolis force An apparent force that seems to deflect a moving object to the right in the northern hemisphere and to the left in the southern hemisphere of the Earth.
Crustal plates Portions of the Earth's crust, which have irregular shapes and sizes and which contact and interact with each other while floating on the asthenosphere.
Cyanobacteria Oxygenic, photosynthetic members of the domain Bacteria, formerly known as blue-green algae.
Cysteine $HSCH_2CH(NH_2)COOH$.
Cytchrome system An electron transport system used in biological oxidation (respiration) that includes iron porphyrin proteins called cytochromes.
Dehydrogenases An enzyme that catalyzes removal or addition of hydrogen.
Denitrification A process in which nitrate is reduced to dinitrogen (N_2), nitrous oxide, and nitric oxide.
Deoxyribonucleic acid A biopolymer consisting of purine and pyrimidine bases, deoxyribose, and phosphate and has genetic information encoded in it.
Desert varnish A manganese- and iron-rich coating on a rock surface.
Desferrisiderophore A siderophore that does not contain ferric iron complexed by it.
Deuteromycetes Fungi that do not form sexual spores.
Diagenesis A process of transformation or alteration of rocks or minerals.
Diatom A Chrysophyte alga grouped with the Bacillarophyceae, that is encased in a siliceous wall.
Diatomaceous ooze A sediment having diatom frustules as a major constituent.
Dimethyl arsinate

$$\begin{array}{l} CH_3AsO(OH) \\ \quad | \\ \quad CH_3 \end{array}$$

The acid form is also known as cacodylic acid.

Dimethylmercury $(CH_3)_2Hg$.
Dimictic lake A lake that turns over twice a year.
Disproportionation reaction A stoichiometric chemical reaction in which part of the reactant undergoes oxidation and the rest undergoes reduction, e.g., $2H_2O_2 \rightarrow 2H_2O + O_2$.
Dithiothreitol $HSCH_2CH(OH)CH_2SH$.
Divergence A separation of two water masses.
DNA Deoxyribonucleic acid.
Dolomite A $Ca(Mg)CO_3)_2$ mineral.
Domain In phylogeny, the highest level of grouping based on cell organization, e.g., in the Prokaryotes, the domains of the Bacteria and the Archaea.
Dunite An ultrabasic rock rich in olivine.
Dystrophic Referring to waters with an oversupply of organic matter that is only incompletely decomposed because of an insufficiency of oxygen, phosphorus, and/or nitrogen.
Earth's core The innermost portion of the Earth, consisting mostly of Fe and some Ni.
Earth's mantle The portion of the Earth overlying the core, containing mainly of O, Mg, and Si with lesser amounts of Fe, Al, Ca, and Na.
Enargite A copper-arsenic sulfide mineral, Cu_3AsS_4.
Endolithic Living inside rock (limestone) as a result of boring into it.
Endosymbiosis Cells that live inside other cells for mutual benefit.
Enrichment culture A culture method that selects for a desired organism(s) by providing special nutrients and/or physical conditions that favor its (their) development; also known as selective culture method.
Entisol An immature desert soil.
Epigenetic Referring to emplacement of a mineral in cracks or fissures of preexisting rock.
Epilimnion The portion of a lake above the thermocline.
Epiphytes Organisms attached to the surface of other living organisms or inanimate objects.
Eukaryotic cell A cell with a true nucleus, mitochondria, and chloroplasts (if photosynthetic).
Euphotic zone The part of a water column that is penetrated by sunlight in sufficient quantity to permit photosynthesis.
Euryhaline Capable of growth over a wide range of salinities.
Eutrophic Referring to a nutrient-rich status of a body of natural water.
Facultative chemolithotroph A bacterium that can grow heterotrophically or chemolithotrophically, depending on growth conditions.
Facultative microorganism A microorganism capable of living with or without oxygen.

Fauna A term used in ecology to denote an assemblage of organisms that may include members of one or more of the following groups: Protozoa, invertebrates, and vertebrates, even though protozoa do not belong to the Animalia in modern systematics.

Fecal pellet Compacted fecal matter packaged in a membrane by the organism that excretes it.

Feldspar A type of mineral consisting of anhydrous aluminosilicates of Na, K, Ca, and Ba.

Fermentation A metabolic process of intramolecular oxidation/reduction operating without an externally supplied terminal electron acceptor; a biochemical disproportionation.

Ferrisiderophore A siderophore that contains ferric iron complexed by it.

Flora A term used in ecology to denote an assemblage of organisms that may include members of one or more of the following groups: prokaryotes, algae, fungi, and plants, even though the first three groups are not considered plants in modern systematics.

Fluorescence microscopy A microscopy method making use of natural or artificial fluorescence of objects upon irradiation with UV light.

Foraminifera Amoeboid protozoa that mostly form a calcareous test (shell) about them; some form tests by cementing sand grains or other inorganic detrital structures to their cell surface (e.g., arenaceous foraminifera).

Fungi Mycelial or, occasionally, single-celled eukaryotic organisms, possessing a cell wall but no chloroplasts; yeasts, molds, mildews, and mushrooms are examples.

Galena A lead sulfide mineral, PbS.

Gangue A term of technical slang that refers to the host rock of an ore that encloses the metal-containing minerals of the ore.

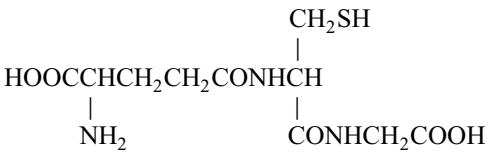
Garnet A silicate mineral of Ca, Mg, Fe, or Mn; it is hard and vitreous.

Generation time The average time required for cell doubling.

Geomicrobiology The study of microbes and the role they have played and are playing in a number of fundamental geologic processes.

Gleying An anaerobic process in some soils involving microbial reduction of ferric iron manifested by a color change from brownish to grayish and development of stickiness; often associated with water logging of soil.

Glutathione



Goethite An iron oxide mineral, $Fe_2O_3 \cdot H_2O$ or α-FeOOH.
Gondwana A former continent encompassing Africa, South America, Australia, Antarctica, and India.
Gram-negative Referring to a differential staining reaction of bacteria in which a counterstain, usually safranin, is retained by the cell.
Gram-positive Referring to a differential staining of bacteria in which the primary stain, crystal violet, is retained by the cell.
Granite Rock of volcanic origin showing coarse crystallization due to slow cooling of the magma from which it arose; granite is rich in quartz and feldspars.
Grandiorite A volcanic rock intermediate between granite and diorite, showing coarse crystallization.
Gravitational water A film of water surrounding pellicular water, that moves by gravity, responds to hydrostatic pressure, and may freeze.
GSH Reduced glutathione.
GSSG Oxidized glutathione.
Guyot Flat-topped seamount.
Gypsum A calcium sulfate mineral, $CaSO_4 \cdot 2H_2O$.
Halophile A microbe that grows preferentially at a high salt concentration.
Hematite An iron oxide mineral, Fe_2O_3.
Heterotroph An organism requiring one or more organic nutrients for carbon and for energy for growth.
Heulandite A type of zeolite mineral.
Histosol Organic soil.
Holozoic Feeding on living cells; predatory.
Homeostasis Maintenance of a state of equilibrium.
Hornblende A type of amphibole mineral.
Humic acid A humus fraction that is acid- and alcohol-insoluble.
Humus In soil a mixture of substances derived from partial decomposition of plant, animal, and microbial remains and from microbial syntheses; in marine sediment of the open ocean, a mixture of substances derived from phytoplankton remains.
Hydrogenase An enzyme catalyzing the reaction $H_2 \Leftrightarrow 2H^+ + 2e$.
Hydrosphere That portion of the Earth's surface that is covered by water; it includes the oceans, seas, lakes, rivers, and groundwater.
Hydrothermal solution A hot, metal-laden solution generated by reaction of water (e.g., seawater) with rock in the lithosphere in regions receiving heat from adjacent magma chambers.
Hygroscopic water A thin film of water covering a soil particle, which never freezes or moves as a liquid.
Hypersthene A type of pyroxene mineral.
Hypha(e) A branch of a mycelium; it is filamentous.
Hypolimnion The portion of a lake located below the thermocline.

Hypophosphite HPO_2^{2-}.
Igneous rock Rock of volcanic or magmatic origin.
Illite A group of micalike clay minerals, that have a three-layered structure like montmorillonite in which Al may substitute for Si and that contain significant amounts of Fe and Mg.
Indigenous organisms Organisms native to a habitat.
Inducible enzyme An enzyme that is formed by a cell only when needed.
Juvenile water Water from within the Earth that had never before reached the Earth's surface.
Kaolinite A type of clay $[Al_4SiO_{10}(OH)_8]$ featuring alternating aluminum oxide and tetrahedral silica sheets.
Karstic Referring to a landscape with sinkholes or cavities due to local dissolution of limestone.
Kazakhstania A former continent encompassing present-day Kazakhstan.
Labradorite A type of feldspar mineral related to plagioclase.
Laterization A soil transformation in which iron and aluminum oxides, silicates, and carbonates are precipitated, cementing soil particles together and thus destroying the porosity of the soil.
Laurasia A former continent encompassing North America, Europe, and most of Asia.
Laurentia A formet continent encompassing most of North America, Greenland, Scotland, and the Chukotski Peninsula of eastern Russia.
Lentic waters Static waters.
Lichen A consortium involving an intimate association of a fungus and a green alga or a cyanobacterium.
Lignin A heteropolymer of units of substituted phenylpropane derivatives; an abundant constituent of wood.
Limestone A type of rock that is rich in $CaCO_3$.
Limonite An amorphous iron oxide mineral, FeOOH or $Fe_2O_3 \cdot nH_2O$.
Lithification A process of rock formation by compaction and/or cementation of sediment.
Lotic waters Flowing waters.
Macrofauna The fauna excluding protozoa and microscopic invertebrates.
Magma Molten rock beneath the Earth's surface.
Mannitol A polyhydric alcohol, that may be formed by reduction of fructose or mannose.
Meddies In oceanography, small closed-current systems of water whose core is more saline than the surrounding water and that exhibit clockwise rotation (*see also* Rings and Anticyclones).
Mercaptoethanol $HOCH_2CH_2SH$.
Mesophile A microorganism capable of growth in a temperature range of 10–45°C (optimal range between 25–40°C).

Mesotrophic Referring to a nutritional state of a natural body of water between oligotrophic and eutrophic.
Metabolism Cellular biochemical activities collectively.
Metabolite A metabolic reactant or product.
Metamorphic rock Rock produced by alteration of igneous or sedimentary rock through action of heat and pressure.
Methanogen A methane-forming bacterium (archeon).
Methanotroph A methane-oxidizing bacterium.
Methylotroph A methanol-oxidizing microbe, that can oxidize methanol but not methane.
Microaerophilic organism An organism that requires a low concentration of oxygen.
Microcosm An experimental setup that approximates important features of a natural environment, but on a small scale that can be manipulated, e.g., a soil or sediment percolation column.
Mineralization In *microbial physiology*, the complete decomposition of an organic compound into CO_2, H_2O, and, if the corresponding elements are present in the organic compound, PO_4^{2-}, NO_3^- or NH_4^+, and SO_4^{2+} or H_2S. In *mineralogy*, the formation of a mineral.
Mitochondria Cytoplasmin organelle of eukaryotic cells in which respiration takes place by which energy is conserved through ATP synthesis.
Mixotroph A bacterium that uses simultaneous inorganic and organic energy sources and/or inorganic or organic carbon sources.
Molydenite A molybdenum disulfide mineral, MoS_2.
Monomethyl arsinate

$$\begin{array}{l} CH_3AsO(OH) \\ \quad\quad | \\ \quad\quad OH \end{array}$$

in acid form.
Monomictic lake A lake that turns over once a year.
Montmorillonite A type of clay mineral $[Al_2Si_4O_{10}(OH)_2 \cdot nH_2O]$ consisting of successive aluminum oxide sheets, each sandwiched between two sheets of silica tetrahedra.
Mycelium A network of hyphae produced by most fungi and some bacteria.
Nepheline A sodium aluminum silicate.
Nitrate ammonification Reduction of nitrate to ammonia via nitrite.
Nitrification A bacterial process in which ammonia is converted to nitrate autotrophically or heterotrophically; some fungi are also capable of heterotrophic nitrification.
Nitrogen fixation A bacterial process in which dinitrogen (N_2) is enzymatically reduced to ammonia.

Nucleic acid A biopolymer containing purines, pyrimidines, pentose or deoxypentose and phosphoric acid found in chromosomes, plasmids, ribosomes, plastids, and cytoplasm of cells.
Nucleotides Polymer units of nucleic acid consisting of a purine or pyrimidine plus pentose or deoxypentose and phosphoric acid.
Ocean eddies Collectively, the oceanic small, closed-current systems: rings, anticyclones, and meddies.
Ocean trench Deep cleft in the ocean floor; a site of subduction of an oceanic crustal plate below a continental plate.
Ochre An iron oxide ore, FeOOH.
Oligotrophic Referring to a nutrient-poor state in a natural body of water.
Olivine A mineral consisting of orthosilicate of magnesium and iron.
Organic soil A soil formed from accumulation of slow and incomplete decomposition of organic matter in a sedimentary environment.
Orogeny Mountain building.
Orpiment An arsenic sulfide mineral, As_2S_3.
Orthoclase A feldspar mineral.
Orthophosphate Monomeric phosphate, H_3PO_4.
Orthosilicate Monomeric silicate, H_4SiO_4.
Oxidative phosphorylation A process of ATP synthesis coupled to electron transport in respiration.
Oxisol A soil type in tropic and subtropic humid climates.
Pangaea A supercontinent including all major continents of today, existing from about 250 to 200 million years ago.
Panspermia Transfer of life in the form of spores from one world (universe, planet) to Earth.
PAPS 3′-Phosphoadenosine phosphosulfate; 3′-phosphoadenosine phosphatosulfate.
Pectinolytic Capable of enzymatic hydrolysis of pectin.
Pedoscope A system of glass capillaries with optically flat sides for insertion into soil and subsequent microscopic inspection for microbial development in the capillary lumen.
Pellicular water A film of water surrounding hygroscopic water that moves by intermolecular attraction and that may freeze.
Peloscope A system of capillaries with optically flat sides for insertion into sediment and subsequent microscopic inspection for microbial development in the capillary lumen.
Pennate geometry Symmetrical about a long and a short axis, in reference to diatoms.
Peptone A mixture of peptides from a digest of beef muscle by pepsin; used in bacterial culture media.
Peridotite An igneous granitoid rock, rich in olivines but lacking in feldspars.

Peroxidase An enzyme that catalyzes the reduction of H_2O_2 by oxidizable organic molecule.
Phagotrophic Consuming whole cells by engulfment (phagocytosis).
Phosphatase An enzyme that catalyzes the hydrolysis of phosphate esters.
Phosphine PH_3.
Phosphite HPO_3^{2-}.
Phosphorite A calcium phosphate mineral; apatite.
Photolithotroph An autotroph that derives its energy from sunlight.
Photophosphorylation A light-dependent process of ATP synthesis associated with photosynthesis.
Photosynthesis A metabolic process using energy form sunlight for the assimilation of carbon in the form of CO_2, HCO_3^-, or CO_3^{2-}.
Phycomycete Aquatic or terrestrial fungus whose vegetative mycelium shows no septation (e.g., *Rhizopus*).
Phytoplankton Photosynthetic plankton.
Plankton Free-floating biota in an aqueous habitat.
Plasmid An extrachromosomal bit of genetic substance (DNA).
Plutonic water Deep, anoxic underground water, likely containing significant amounts of sulfate and/or chloride.
Podzolic soil A type of spodosol associated with humid, temperate climates; a naturally acidic forest soil.
Pregnant solution A metal-laden effluent from an ore-leaching operation.
Primary producers Organisms that transform (fix) CO_2 into organic carbon; include photo- and chemolithotrophs.
Prokaryotic cell A cell lacking a true nucleus, mitochondria, and chloroplasts.
Proteolytic Referring to enzymatic hydrolysis of proteins.
Psychrophile A microorganism capable of growth in a temperature range from slightly below 0 to 20°C (optimum at 15°C or below).
Psychrotolerant Capable of surviving but not growing at a temperature in the psychrophilic range.
Psychrotroph A microorganism capable of growth in a temperature range of 0–30°C (optimum about 25°C).
Pure culture A microbial culture that consists of one and only one species or strain.
Purines A group of organic bases having a purine ring structure in common.
Pyridine nucleotide Nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate; a hydrogen-carrying coenzyme.
Pyrimidines A group of organic bases having the pyrimidine ring structure in common.
Pyrite (iron) An iron disulfide mineral, FeS_2.
Pyroxene A ferromagnesian mineral with silica tetrahedra linked in single chains and cross-linked mainly by Ca, Mg, and Fe.

Quartzite A metamorphic rock derived from sandstone.
Radiolarian ooze A sediment having radiolarian tests as a major constituent.
Red-bed deposit A sedimentary deposit rich in ferric oxide; first appeared after the atmosphere of the Earth became oxidizing.
Respiration Biological oxidation utilizing an electron transport system that may operate with either oxygen or another external, reducible inorganic or organic compound as terminal electron acceptor.
Reverse electron transport The transfer of electrons by an electron transport system against the redox gradient, requiring the input of metabolic energy.
Rhizosphere The zone in soil that surrounds the root system of a plant, where special environmental conditions may prevail as a result of root secretion or uptake of specific inorganic and/or organic substances. It represents a special habitat for some microbes.
Rhodanese An enzyme capable of catalyzing the reaction $CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^-$ and the reductive cleavage of $S_2O_3^{2-}$.
Rhodochrosite A mineral form of $MnCO_3$.
Rhyolite An igneous rock rich in plagioclase feldspar.
Ribonucleic acid (RNA) Heteropolymer consisting of purine and pyrimidine bases, ribose, and phosphoric acid. Different forms of RNA may serve as templates in protein synthesis (messenger RNA), as amino acid transfer RNA (to locate position in peptide chain determined by messenger RNA), and as part of the structure of ribosomes.
Ribosome Submicroscopic, intracellular particle that consists of different ribonucleic acids and proteins and is part of the protein synthesizing system of cells.
Ribulose bisphosphate carboxylase/oxygenase An enzyme that catalyzes carboxylation or oxygenation of ribulose diphosphate in many autotrophic bacteria and in algae and plants.
Rings In oceanography, a small closed-current system, with a diameter as great as 300 km and depth as great as 2 km and a core of cold water surrounded by warmer water and rotating counterclockwise.
RNA Ribonucleic acid.
Rock Massive, solid inorganic matter, usually consisting of two or more intergrown minerals.
Rusticyanin A copper-containing, periplasmic enzyme that is involved in Fe^{2+} oxidation in *Thiobacillus ferrooxidans*.
Saccharolytic Capable of enzymatic hydrolysis or fermentation of sugars.
Salinity A measure of the salt content of seawater based on its chlorinity.
Salt dome The cap rock composed of anhydrite, gypsum, and calcite at the top of a salt plug; a geologic formation.
Sandstone A rock formed from compacted and cemented sand.
Saponite A montmorillonite type of clay in which Mg replaces Al.

Saprozoic Feeding on dead organic matter.
Satellite microorganism An organism not identical to the dominant organism in a mixed culture, which will give rise to distinctive colonies on appropriate solid medium.
Sclerotium A vegetative, resting, food storage body in higher fungi, composed of a compact mass of hardened mycelium. Plural: sclerotia.
Sediment Finely divided mineral and organic matter that has settled to the bottom in a body of water.
Sedimentary rock Rock formed from compaction and/or cementation of sediment.
Seismic activity Earth tremors.
Shale A laminate sedimentary rock formed from mud or clay.
Sheath In bacteriology, an organic tubular structure around some bacterial organisms.
Siderite A mineral form of $FeCO_3$.
Siderophore An organic iron-chelating substance produced by certain microbes.
Silica Silicon dioxide; quartz and opal are examples.
Silicate A salt of silicic acid; a mineral containing silicate.
Slime molds A group of eukaryotic microorganisms that have a life cycle including a motile swarmer stage and an aggregational phase, which may be multinucleate, leading to formation of a sessile fruiting body.
Sodium azide NaN_3, an inhibitor of cytochrome oxidase.
Soil horizon A soil stratum as seen in a soil profile.
Soil profile A vertical section through soil.
Solfatara Fumarolic hot spring that yields sulfuretted water.
Spent culture medium Culture medium after microbial growth has taken place in it.
Spodosol Forest soil type in temperate climates.
Stenohaline Capable of growth in only a narrow range of salinities.
Stromatolite A laminated structure formed from filamentous organisms that grew in mats that either entrapped inorganic detrital material or formed $CaCO_3$ deposits in which the organism became embedded; the organisms in modern stromatolites are most commonly cyanobacteria; in Precambrian stromatolites, the organic remains have frequently disappeared owing to replacement by silica.
Subduction A process in which the edge of an oceanic crustal plate slips under a continental plate manifested in the form of deep ocean trenches.
Substrate-level phosphorylation A process of ATP synthesis involving high-energy phosphate bond formation on the substrate being oxidized.
Sulfate-reducing bacteria (SRB) Bacteria that convert sulfate to sulfide as part of a respiratory process; includes members of the domains Bacteria and Archaea.
Sulfhydryl compound An organic compound with one or more −SH functional groups.

Superoxide dismutase An enzyme that catalyzes the disproportionation of superoxide (O_2^-) into H_2O_2 and O_2.
Syngenetic Referring to deposition of an ore mineral contemporaneously with the enclosing sediment or rock.
Synergism The interaction of two or more microorganisms, resulting in a reaction that none of the organisms could carry out alone.
Talc A hydrous magnesium silicate mineral.
Tectonic activity Interaction of crustal plates of the Earth.
Teichoic acid A glycerol- or ribitol-based polymeric constituent of the cell walls of gram-positive bacteria.
Tetrathionate $S_4O_6^{2-}$.
Thermocline A zone in a water column with a steep temperature gradient.
Thermophiles Bacteria that grow at temperatures above 45°C; some have been shown capable of growing above the boiling point of water when under pressure.
Thiobacilli Gram-negative rod-shaped bacteria, mostly chemolithotrophic, that can use H_2S, S°, or $S_2O_3^{2-}$ as energy sources.
Thiosulfate $S_2O_3^{2-}$.
Todorokite A complex manganese(IV) oxide.
Transduction A method of transfer of genetic information between bacteria, involving a bacterial virus as the transmitting agent.
Transpiration Loss of water by evaporation through the stomata (pores) of leaves.
Travertine A porous limestone that may be formed by rapid $CaCO_3$ precipitation by cyanobacteria.
Tricarboxylic acid cycle A cyclic sequence of biochemical reactions in which acetate is completely oxidized in one turn of the cycle.
Trithionate $S_3O_6^{2-}$.
Trophosome A structure in the coelomic cavity of some vestimentiferan worms consisting of a mass of active symbiotic hydrogen sulfide–oxidizing bacteria, which share with the worm the carbon they fix chemoautotrophically.
Tundra soil A soil type occurring at high northern latitudes.
Turbidity current A strong ocean current of a sediment suspension; may exert scouring action as it moves over rock surfaces.
Ultramafic rock An igneous rock, usually rich in olivine and pyroxenes.
Upwelling An upward movement of a mass of deep, cold ocean water, which may bring nutrients (nitrate, phosphate) into surface waters.
Vermiculite A micaceous mineral.
Wad A complex manganese(IV) oxide.
Wadi *See* Arroyo.
Water potential A measure of water availability, for instance in soil.
Weathering A breakdown process of rock.

Wollastonite A calcium silicate mineral, $CaSiO_3$.
Zeolite A hydrated silicate of aluminum containing alkali metals.
Zooplankton Nonphotosynthetic plankton.
Zygospore A sexual spore formed by certain algae and fungi.