LBNL-42595 (2003)

BIOREMEDIATION

OF METALS AND RADIONUCLIDES

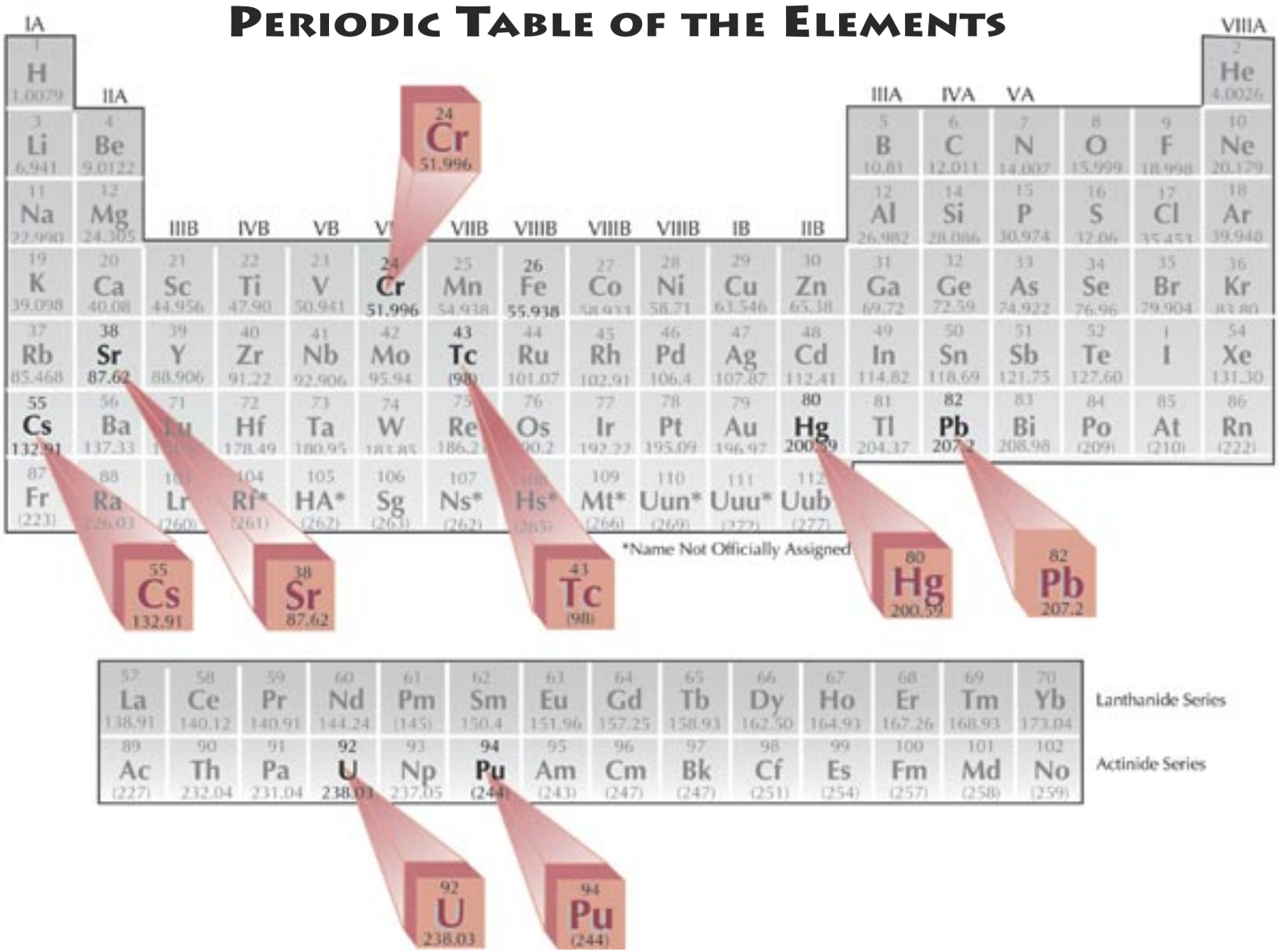
...WHAT IT IS AND HOW IT WORKS

2ND EDITION
2003

A NABIR Primer

Prepared for the Natural and Accelerated Bioremediation Research Program, Office of Biological and Environmental Research, Office of Science, U.S. Department of Energy.

Periodic Table of the Elements



The elements highlighted in this table are some of the most common constituents of contaminant waste at DOE sites.

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Table of Contents

CONTRIBUTORS 1

FOREWORD 3

SECTION I:

THE PROBLEM: METALS AND RADIONUCLIDES AT DOE SITES 5

Our Nation's Cold War Legacy 5

Overall Environmental Restoration Objectives 5

The Focus on Radionuclides and Metals 6

SECTION II:

WHAT IS BIOREMEDIATION? 9

Introduction 9

Which Bioremediation Technology Should Be Used? 10

Feature: Soils and Sediments — From a Microbe's Perspective 12

Feature: Contaminant Plumes: Migration of Hazardous Waste in the Subsurface 16

SECTION III:

METALS AND RADIONUCLIDES FOUND AT CONTAMINATED SITES 19

Radionuclides 19

Feature: Opposites Attract: Valences, Bonds, and Redox Reactions 20

Metals 25

SECTION IV:

A LOOK AT MICROBIAL METABOLISM 29

Basic Microbial Metabolic Processes 30

Feature: Who's Out There? Microbial Species that Live in the Subsurface 31

Microbial Respiration 36

Feature: Exploring the Diversity of Iron(III)-Reducing Bacteria in Subsurface Sediments 39

Feature: Genomics, Proteomics, and Bioremediation 40

SECTION V:

MICROBIAL PROCESSES AFFECTING THE BIOREMEDIATION OF METALS AND RADIONUCLIDES 45

Biotransformation 46

Bioaccumulation and Biosorption 49

Siderophore-Mediated Uptake by Microorganisms 50

Microbes and Synthetic Organic Chelators 51

SECTION VI:

FIELD RESEARCH ON BIOREMEDIATION OF METALS AND RADIONUCLIDES . . 53

Field Research Sites 53

Feature: Mathematical Modeling of Subsurface Processes 56

Examples of Field Research on Bioremediation of Metals and Radionuclides 56

Feature: The Importance of Scale 59

Feature: Environmental Monitoring: Keeping an Eye on the Subsurface 60

Rewards of Field Research 62

The Future of Natural and Accelerated Bioremediation Research 62

GLOSSARY 63

ACRONYMS 71

REFERENCES 73

BIOREMEDIATION WEB SITES 77

A Special Thank You...

The NABIR Program wishes to acknowledge and thank a select group of people who dedicated their time to thoughtful and insightful review of the second edition of the NABIR Primer, *Bioremediation of Metals and Radionuclides . . . What It Is and How It Works*. Without their time and effort, this publication would not be the resource it is today:

Scott Fendorf
Stanford University

Allan Konopka
Purdue University

Joel Kostka
Florida State University

Derek Lovley
University of Massachusetts

Blaine Metting
Pacific Northwest National Laboratory

Brent Peyton
Washington State University

Judy Wall
University of Missouri

David Watson
Oak Ridge National Laboratory

David White
University of Tennessee

Ray Wildung
Pacific Northwest National Laboratory

A PDF version of this publication can be found at: http://www.lbl.gov/NABIR/generalinfo/primersguides.html

Contributors

The 2nd edition of the NABIR primer greatly benefited from contributions from the following scientists:

Calvin Ainsworth	Pacific Northwest National Laboratory
Todd Anderson	U. of Massachusetts
Robert Anex	U. of Oklahoma
Tamar Barkay	Rutgers University
Diane Blake	Tulane University
Craig Brandt	Oak Ridge National Laboratory
Harvey Bolton	Pacific Northwest National Laboratory
Fred Brockman	Pacific Northwest National Laboratory
Bill Burgos	Penn. State University
Susan Clark	Washington State University
John Coates	U. California at Berkeley
Pam Conrad	U. Southern California
Craig Criddle	Stanford University
Michael Daly	Uniformed Serv. U. of the Health Sci.
Scott Fendorf	Stanford University
Jim Fredrickson	Pacific Northwest National Laboratory
Carol Giometti	Argonne National Laboratory
Yuri Gorby	Pacific Northwest National Laboratory
Baohua Gu	Oak Ridge National Laboratory
Larry Hersman	Los Alamos National Laboratory
Bruce Honeyman	Colorado School of Mines
Jack Istok	Oregon State University
Peter Jaffe	Princeton University
Phil Jardine	Oak Ridge National Laboratory
Ken Kemner	Argonne National Laboratory
Allan Konopka	Purdue University
Joel Kostka	Florida State University
Lee Krumholz	U. of Oklahoma
Denise Lach	Oregon State University
Stuart Levy	Tufts University
Mary Lipton	Pacific Northwest National Laboratory
Jon Lloyd	U. of Manchester
Phil Long	Pacific Northwest National Laboratory
Derek Lovley	U. of Massachusetts
Yi Lu	U. of Illinois
Terry Marsh	Michigan State University
A.C. Matin	Stanford University
Ken Nealson	U. of Southern California
Mary Neu	Los Alamos National Laboratory
Heino Nitsche	Lawrence Berkeley National Laboratory
Brent Peyton	Washington State University
Margaret Romine	Pacific Northwest National Laboratory
Anne Summers	U. of Georgia
Jim Tiedje	Michigan State University
Tetsu Tokunaga	Lawrence Berkeley National Laboratory
Judy Wall	U. of Missouri
Jiamin Wan	Lawrence Berkeley National Laboratory
David Watson	Oak Ridge National Laboratory
David White	U. of Tennessee
Amy Wolfe	Oak Ridge National Laboratory
Brian Wood	Oregon State University
John Zachara	Pacific Northwest National Laboratory
Jizhong Zhou	Oak Ridge National Laboratory

Contributors from the Department of Energy, (DOE), the Office of Biological and Environmental Research (BER), and the NABIR Program Office at Lawrence Berkeley National Laboratory (LBNL):

Maria Atkinson, *LBNL*
Paul Bayer, *DOE-BER*
Daniel Drell, *DOE-BER*
Brendlyn Faison, *DOE-BER*
Terry C. Hazen, *LBNL*
Arthur Katz, *DOE-BER*
Julie McCullough, *LBNL*
Anna Palmisano, *DOE-BER*
Sherry Seybold, *LBNL*
Linda Wuy, *LBNL*

Foreword

This primer is intended for people interested in environmental problems of the U.S. Department of Energy (DOE) and in their potential solutions. It will specifically look at some of the more hazardous metal and radionuclide contaminants found on DOE lands and at the possibilities for using bioremediation technology to clean up these contaminants. The second edition of the primer incorporates recent findings by researchers in DOE's Natural and Accelerated Bioremediation Research (NABIR) Program.

Bioremediation is a technology that can be used to reduce, eliminate, or contain hazardous waste. Over the past two decades, it has become widely accepted that microorganisms, and to a lesser extent plants, can transform and degrade many types of contaminants. These transformation and degradation processes vary, depending on the physical-chemical environment, microbial communities, and nature of the contaminant. This technology includes intrinsic bioremediation, which relies on naturally occurring processes, and accelerated bioremediation, which enhances microbial degradation or transformation through the addition of nutrients (biostimulation) or inoculation with microorganisms (bioaugmentation).

Over the past few years, interest in bioremediation has increased. It has become clear that many organic contaminants such as hydrocarbon fuels can be degraded to relatively harmless products such as CO_2 (the end result of the degradation process). Waste water managers and scientists have also found that microorganisms can interact with metals and convert them from one chemical form to another. Laboratory tests and ex situ bioremediation applications have shown that microorganisms can change the valence, or oxidation state, of some heavy metals (e.g., chromium and mercury) and radionuclides (e.g., uranium) by using them as electron acceptors. In some cases, the solubility of the altered species decreases and the contaminant is immobilized in situ, i.e., precipitated into an insoluble salt in the sediment. In other cases, the opposite occurs — the solubility of the altered species increases, increasing the mobility of the contaminant and allowing it to be more easily flushed from the environment. Both of these kinds of transformations present opportunities for bioremediation of metals and radionuclides — either to lock them in place, or to accelerate their removal. DOE's goal is to reduce the risk and related exposure to ground water, sediment, and soil contamination at Department of Energy facilities.

Subsurface bioremediation of metals and radionuclides at the site of contamination (in situ bioremediation) is not yet in widespread use. However, successful in situ applications of bioremediation to petroleum products and chlorinated solvents provide experience from which scientists can draw. Taken together, the accomplishments in these areas have led scientists and engineers to be optimistic about applying this technology to the mixtures of metals and radionuclides that are found at some of the most contaminated DOE sites.

This primer examines some of the basic microbial and chemical processes that are a part of bioremediation, specifically the bioremediation of metals and radionuclides. The primer is divided into six sections, with the information in each building on that of the previous. The sections include features that highlight topics of interest and provide background information on specific biological and chemical processes and reactions.

The first section briefly examines the scope of the contamination problem at DOE facilities. The second section gives a summary of some of the most commonly used bioremediation technologies, including successful in situ and ex situ techniques. The third discusses chemical and physical properties of metals and radionuclides found in contaminant mixtures at DOE sites, including solubility and the most common oxidation states in which these materials are found. The fourth section is an overview of the basic microbial processes that occur in bioremediation. The fifth section looks at specific in situ bioremediation processes that can be used on these contaminant mixtures. The primer concludes with examples of field research on bioremediation of metals and radionuclides.

[1] The NABIR Program is responsible for the development of this primer. NABIR focuses on the in situ bioremediation of metals and radionuclides in the subsurface below the root zone. However, this primer discusses a broader range of remediation technologies than the program supports, giving its readers an overall context for bioremediation technology.

THE PROBLEM:
METALS AND RADIONUCLIDES AT DOE SITES

OUR NATION'S COLD WAR LEGACY

For more than 50 years the United States has used nuclear energy for both civilian and military purposes. This use resulted in the creation of a vast network of facilities across the nation engaged in research, development, production, and testing of nuclear materials. Since most of this nuclear material has been related to weapons production, this network is referred to as the nuclear weapons complex. The U.S. Department of Energy (DOE) and its predecessor agencies (the Atomic Energy Commission and the Energy Research and Development Agency) have primary responsibility for the nuclear weapons complex. A civilian agency has always been responsible for this nuclear weapons network.

With the end of the Cold War threat in the early '90s and the subsequent shutdown of all nuclear weapons production reactors in the United States, DOE has shifted its emphasis to remediation, decommissioning, and decontamination of the immense volumes of contaminated water and soils, and the over 7,000 structures spread over 120 sites (7,280 square kilometers) in 36 states and territories. DOE's environmental legacy includes 1.7 trillion gallons of contaminated ground water in 5,700 distinct plumes, 40 million cubic meters of contaminated soil and debris, and 3 million cubic meters of waste buried in landfills, trenches, and spill areas (*Linking Legacies Report*, January 1997). The first few years of cleanup have mainly involved cataloging and preliminary characterization. The Department of Energy currently has more than 350 cleanup projects, with a total life-cycle cost of $220 billion and a completion schedule of more than 70 years. Without major technical breakthroughs, the cost is expected to rise to $300 billion, an increase of over 36%, and could go much higher (*Status Report on Paths to Closure*, 2000). The DOE cleanup of the Cold War legacy wastes is the largest program of its kind ever undertaken by the United States. Environmental stewardship of these sites may require long-term monitoring and maintenance for hundreds of years. Long-term stewardship can be defined as the physical controls, institutions, information, and other mechanisms needed to ensure protection of people and the environment.

OVERALL ENVIRONMENTAL RESTORATION

A key mission of the Department of Energy is to "permanently and safely dispose of the radioactive wastes generated from the production of nuclear weapons during the Cold War" (*Environmental Quality: Long Term Stewardship*, 2002). DOE's Office of Environmental Management (EM) has the major responsibility for this enormous cleanup effort and has identified five major environmental restoration needs (*EM Research and Development Program Plan*, October 1998):

(1) The most cost-effective remediation plans require a complete and accurate understanding of the inventory, distribution, and movement of contaminants in the vadose (unsaturated) zone and the saturated zone. Improved analytical tools, monitoring devices for use in situ, understanding of permeability patterns, and tools to predict ground water flow and transport are required to characterize and quantify these contaminants.

(2) The ability to contain or stabilize leaks and buried waste hot spots in situ requires resolution of problems in several areas. Improved surface barrier systems are needed to provide effective containment of leaking landfills, trenches, tanks, and high-concentration plumes. Methods are needed to stabilize buried wastes in situ to prevent leaching

and contamination of the vadose zone. Cover systems that provide robust waste isolation over a range of climatic conditions and extreme events for periods of over 100 years are necessary for many applications. Finally, in situ treatment barriers need to be developed to provide effective remediation of dispersed contaminant plumes.

(3) The ability to treat or destroy mobile contaminants in situ is dependent on the resolution of problems in several areas. Biologically based treatment methods are needed for remediation of low to moderate concentrations of organic solvents in sediments and ground water. Chemical treatment technologies to destroy or immobilize highly concentrated contaminant sources (metals, radionuclides, explosive residues, and solvents) in the vadose and saturated zones are required to increase remediation rates. Finally, improved deep drilling technology is required to provide access to deep contaminant plumes for sampling, retrieval, and delivery activities.

(4) Highly radioactive, explosive, and pyrophoric wastes pose unacceptable risks to remediation workers during retrieval and treatment. The capability for on-site characterization and remote retrieval of these hot spots that are not amenable to in situ treatment must be developed.

(5) In order to obtain regulator and stakeholder acceptance of contaminant, stabilization, and treatment technologies in remediation plans, methods to validate and verify containment and treatment system performance and integrity must be developed.

THE FOCUS ON RADIONUCLIDES AND METALS

The Natural and Accelerated Bioremediation Research (NABIR) Program of DOE's Office of Biological and Environmental Research addresses some of DOE's environmental restoration needs by conducting basic research on bioremediation, especially as it relates to radionuclides and metals in subsurface environments. The interdisciplinary research being funded by the program specifically focuses on one or more components in several of the above five need areas. For example, research in the NABIR Program will lead to improved monitoring tools (area 1), in situ treatment technologies (area 2), and treatments to immobilize wastes (area 3).

The rationale for basic research on radionuclides and metals is illustrated by a review of DOE contaminants by waste site and facility (Riley et al., 1992). This review of DOE chemical contaminants and mixtures is one of the few comprehensive comparisons of DOE contaminants; it shows that more than 50% of the facilities and 35% of the waste sites have radionuclide and metal contamination. In soils and sediments, radionuclides and metals are the highest frequency classes of contamination by waste site and the 3rd and 4th highest frequency classes by facility (Figure 1.1). The first two classes by facility (fuel and chlorinated hydrocarbons) are technologically further advanced in the development of cost-effective and efficient solutions. Remediation of radionuclides and metals currently requires greater research emphasis to support technology development.

Metals and radionuclides also dominate ground water contaminants at DOE facilities, with more than 60% having these types of waste (Figure 1.2). Metals and radionuclides also are the highest frequency compound class by waste site, with more than 50% having these contaminants. The only contaminants exceeding the frequency of metal and radionuclide contamination in ground water are chlorinated hydrocarbons, some of which are being treated with existing technologies.

The need for basic research to focus on metals and radionuclides is further underscored by the recognition that radionuclides are uniquely a DOE problem. Because nuclear production was carried out by DOE at DOE sites, it has not received the research attention or funding by other government agencies that solvents, fuels, and a few of the metal contaminants have received. A thorough understanding of the biological, chemical, and physical factors that influence subsurface mobilization and immobilization of radionuclides and metals is needed. This knowledge will allow environmental professionals to enhance, contain, and predict long-term stability of these contaminants in the subsurface and the risk of their migration into surface or ground water. Studies supported by NABIR will not only facilitate our overall understanding of subsurface environments, but could also potentially save hundreds of millions of dollars in cleanup costs and support long-term stewardship of DOE sites.

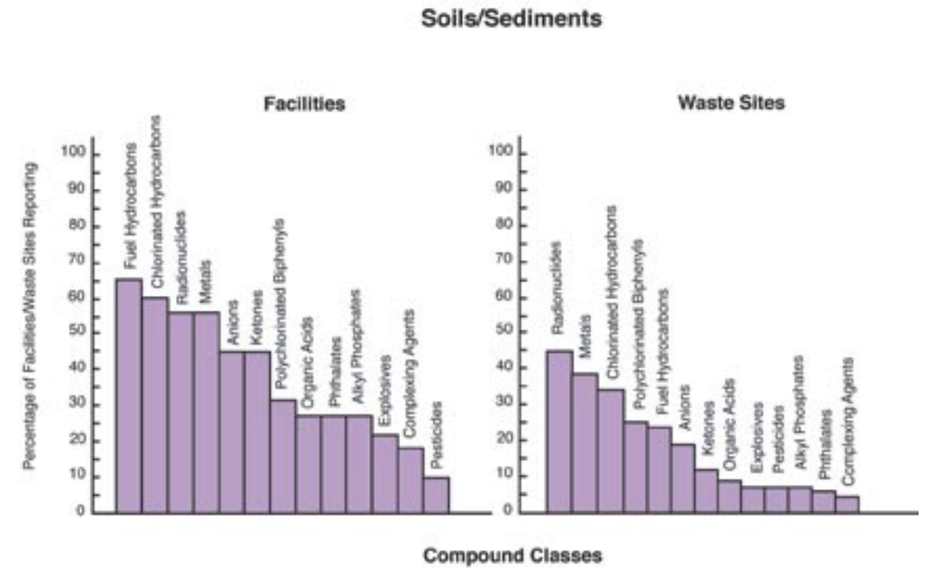

Figure 1.1. Distribution of compound classes in soils and sediments at 18 DOE facilities and 91 waste sites (Riley et al., 1992).

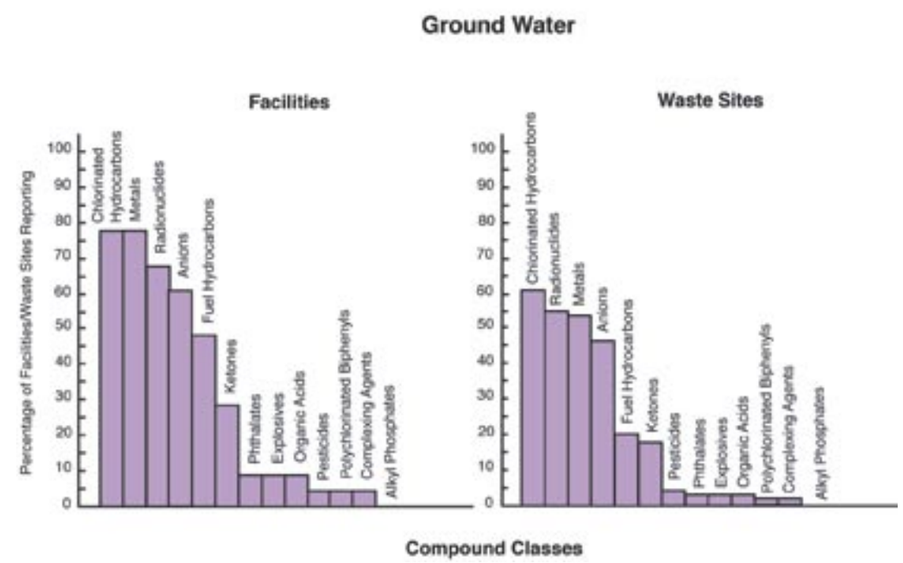

Figure 1.2. Distribution of compound classes in ground water at 18 DOE facilities and 91 waste sites (Riley et al., 1992).

WHAT IS BIOREMEDIATION?

INTRODUCTION

Bioremediation technology uses microorganisms to reduce, eliminate, contain, or transform to benign products contaminants present in soils, sediments, water, or air. Bioremediation is not a new technology. Both composting of agricultural material and sewage treatment of household waste are based on the use of microorganisms to catalyze chemical transformation. Such environmental technologies have been practiced by humankind since the beginning of recorded history. Evidence of kitchen middens and compost piles dates back to 6000 B.C, and the more "modern" use of bioremediation began over 100 years ago with the opening of the first biological sewage treatment plant in Sussex, UK, in 1891. However, the word "bioremediation" is fairly new. Its first appearance in peer-reviewed scientific literature was in 1987 (Hazen, 1997).

The last 15 years have seen an increase in the types of contaminants to which bioremediation is being applied, including solvents, explosives, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Now, microbial processes are beginning to be used in the cleanup of radioactive and metallic contaminants, two of the most common and most recalcitrant components of hazardous waste at DOE sites (see Section I).

This primer looks at the possibilities for in situ bioremediation of radionuclides and metals, which often reside in contaminant plumes in subsurface environments. Of particular interest are the metals chromium and mercury, and the radionuclides uranium, technetium, and plutonium.

The term "mixed waste" refers to radioactive waste that contains organic compounds as well as radionuclides. Although organic components are a part of mixed waste at DOE sites, they are not the focus of this primer. Certain organic compounds, however, can play a central role in metal and radionuclide bioremediation strategies. The synthetic chelators ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) were commonly used as cleaning agents during industrial processing of nuclear fuels at DOE and have formed stable, soluble complexes with certain metals and radionuclides in the subsurface. These chelators may be inherently toxic, and when combined with heavy metals as metal–chelator complexes, they are even more toxic and difficult to clean up. The increased solubility of the metal–chelator complex also allows these metals and radionuclides to move much farther in the subsurface than normal, thereby increasing their probability of reaching risk receptors (drinking water wells and surface waters, e.g., rivers).

Many remediation technologies exist to treat hazardous waste. One of the most common has been pump and treat (extraction and then treatment of the dissolved contaminant by either physical, chemical, or biological processes). Pump and treat is often applied in the remediation of industrial solvents such as trichloroethylene (TCE), which was used to degrease metal surfaces ranging from nuclear target elements to computer components.

Extraction processes do have some major disadvantages. Subsurface sediment and rock formations are heterogeneous, and this lack of uniformity can cause uneven or low-velocity flow patterns. Therefore, it can take a long time to flush contamination out of such heterogeneous formations. Moreover, organic contaminants such as TCE may form dense liquid phases that do not mix well with water. Slow rates of contaminant desorption from particles and diffusive transport may also limit extraction. Some contaminants, such as PCBs, tend to adsorb tightly to mineral surfaces of clays or to soil organic matter. This adsorption can slow extraction, and it may take decades before enough contaminant is removed to make a site safe. Also, bringing the contaminants up to the surface can increase health and safety

risks for cleanup workers, the environment, and the public.

Bioremediation is an alternative to traditional remediation technologies such as landfilling or incineration. Bioremediation depends on the presence of the appropriate microorganisms in the correct amounts and combinations and on the appropriate environmental conditions. Although prokaryotes — Bacteria and Archaea — are usually the agents responsible for most bioremediation strategies, eukaryotes such as fungi and algae also can transform and degrade contaminants. Microorganisms already living in contaminated environments are often well-adapted to survival in the presence of existing contaminants and to the temperature, pH, and oxidation–reduction potential of the site. These indigenous microbes tend to utilize the nutrients and electron acceptors that are available in situ, provided liquid water is present. The bulk of subsurface microbial populations are associated with the solid phase. Water acts as a vehicle to transport both microorganisms and dissolved substances, including contaminants and their breakdown products.

Bioremediation works by either transforming or degrading contaminants to nonhazardous or less hazardous chemicals. These processes are called, respectively, biotransformation and biodegradation. Biotransformation is any alteration of the molecular or atomic structure of a compound by microorganisms. Biodegradation is the breaking down of an organic substance by microorganisms into smaller organic or inorganic components. Biodegradation may proceed all the way to mineralization — the complete biodegradation of an organic contaminant into inorganic constituents such as carbon dioxide (or, in some cases, methane), mineral salts, and water.

Unfortunately, metals and radionuclides cannot be biodegraded. However, microorganisms can interact with these contaminants and transform them from one chemical form to another by changing their oxidation state through the adding of (reduction) or removing of (oxidation) electrons.[1] In some bioremediation strategies, the solubility of the transformed metal or radionuclide increases, thus increasing the mobility of the contaminant and allowing it to more easily be flushed from the environment. In other strategies, the opposite will occur, and the transformed metal or radionuclide may precipitate out of solution, leading to immobilization. Both kinds of transformations present opportunities for bioremediation of metal and radionuclides in the environment — either to immobilize them in place or to accelerate their removal.

WHICH BIOREMEDIATION TECHNOLOGY SHOULD BE USED?

In situ bioremediation refers to below-ground methods applied at the site of contamination. Ex situ is defined as "in a position or location other than the natural or original one." Ex situ bioremediation usually refers to aboveground treatment in which soils have been excavated and washed, or water or sediments have been extracted from the subsurface and then decontaminated.

There are a number of ex situ and in situ bioremediation methods currently available. Ex situ methods have been around longer and are better understood, and they are easier to contain, monitor, and control. However, in situ bioremediation has several advantages over ex situ techniques. In situ treatment is useful for contaminants that are widely dispersed in the environment, present in dilute concentrations, or otherwise inaccessible (e.g., due to the presence of buildings or structures). Another key application of bioremediation is at the forefront of a contaminant plume where a permeable "biobarrier" can be established. In situ bioremediation can be less costly and less disruptive than ex situ treatments because excavation and removal are not required. Moreover, exposure of site workers to hazardous contaminants during in situ treatment is minimal.

A brief overview of several existing bioremediation strategies follows. These methodologies are not mutually exclusive. Depending on the type of contaminant problem, several bioremediation strategies can be used in combination with one another and/or with more traditional physical and chemical remediation techniques.

[1] Microorganisms can do much more than biotransform contaminants. They can also influence contaminant behavior by changing the acidity of the system in the immediate vicinity of the contaminant, or by altering the form of organic compounds that influence radionuclide and metal mobility.

Intrinsic Bioremediation

Intrinsic bioremediation occurs in situ and relies on naturally occurring biological processes carried out by indigenous microorganisms. Intrinsic bioremediation is a component of natural attenuation, which includes physical and chemical processes. Cleanup activities that rely on natural attenuation to reduce contaminant levels and monitoring to determine the remedial effectiveness are referred to as "monitored natural attenuation." Intrinsic bioremediation was first observed several years ago at sites of petroleum hydrocarbon contamination. The pollutants were being biodegraded by the naturally occurring microorganisms at rates fast enough to stop or reduce contaminant spread. To establish that intrinsic bioremediation is actually occurring at a sufficient rate in the subsurface, contaminant plume size and associated microbial activity (biodegradation and/or biotransformation) must be measured over a period of time. At present, intrinsic bioremediation is mainly accepted for petroleum hydrocarbons and, to a limited degree, chlorinated hydrocarbons such as TCE. However, promising results have been obtained with intrinsic bioremediation of selenium-polluted agricultural drainage water in marshlands. It is possible that recent advances in the understanding of the microbiology and geochemistry of sites contaminated with other hazardous and/or radioactive metals will lead to determination of the viability of using natural attenuation for the cleanup of these environments.

Biostimulation and Bioaugmentation

Biostimulation is the addition of nutrients (usually sources of carbon, nitrogen, and/or phosphorus), oxygen, or other electron donors or acceptors. These amendments serve to increase the number or activity of naturally occurring microorganisms available for bioremediation. Amendments can be added in either liquid or gaseous form, via injection. Liquids can be injected into shallow or deep aquifers to stimulate the growth of microorganisms involved in bioremediation (Figure 2.1). Biosparging is a type of soil venting, where air or other gases are injected below the ground into saturated sediments to minimize volatilization of contaminants, such as TCE.

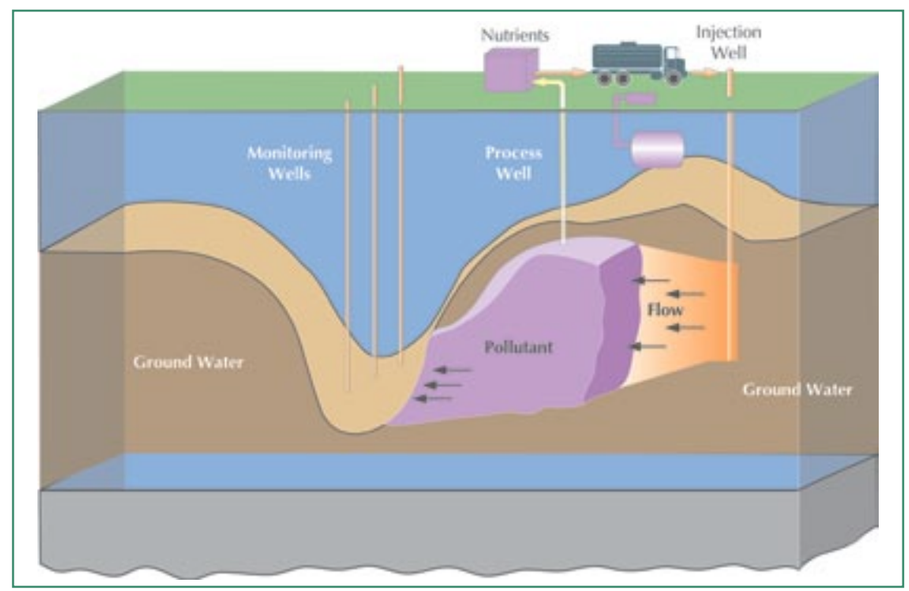

Figure 2.1. In situ treatment of contaminated ground water. Contaminated ground water is pumped to the surface and mixed with nutrients, then injected upgradient of the contaminant plume to biostimulate degradation of the contaminant in situ by the indigenous microbes.

SOILS AND SEDIMENTS — FROM A MICROBE'S PERSPECTIVE

Soils and sediments are a heterogeneous assemblage of solids, liquids, and gases. They contain inorganic and organic material ranging in size from clays (<0.002 mm in particle diameter) to silt (0.002–0.05 mm), to sand (0.05–2 mm), to gravel (>2 mm) or rock. The inorganic component includes quartz (SiO_2) and feldspars ($KAlSi_3O_8$ or $NaAlSi_3O_8$), and other minerals as well as clay minerals such as kaolinite [$Si_6Al_4O_{10}(OH)_8$] and montmorillonite [$M_x(Al,Fe^{2+},Mg)_4Si_8O_{20}(OH)_4$, where M is a metal cation]. Soil or sediment minerals may have specific surface areas, including both external and internal surfaces, of 10 to 1,000 m^2/g.

Natural organic matter (NOM) and humus are synonymous. The structure of NOM varies, but is usually enriched in aromatic hydroxyl groups (–OH) and carboxylic acids (–COOH), which are acidic. Other functionalities such as aliphatic–OH groups are neutral; nitrogen-containing groups such as amines and amides are basic. The acidic groups are most likely to influence the behavior of metals in subsurface environments.

Soil and sediment voids may be occupied by liquids (usually water) and/or by gases (usually air). The saturated zone is a geologic layer in which the fractures and pores are filled with water. The unsaturated zone, or "zone of aeration," above the water table is called the vadose zone. The vadose zone is not entirely dry — water may exist in films on particle surfaces and within the interstices (micropores) of soil particles. This moisture is almost impossible to remove and reflects the hydrogen bonding between water and hydrophilic groups in soils.

Chemical reactions within soils and sediments include ion association, ion exchange, complexation, multivalent ion hydrolysis (or breakdown of complexes), oxidation, reduction, partial or complete degradation of organics, crystallization, sorption, and solubilization/dissolution. These activities do not proceed in a homogenous manner. For example, deposition of solutes may occur fairly evenly across a soil surface, or may instead proceed at nucleation sites formed by recently sorbed solutes. Reactions involve transport of solutes through the bulk liquid (soil solution), across a liquid film at the solid–liquid interface, and within a liquid-filled macropore.

Microorganisms may live at the particle–fluid interface in soils and sediments, even within very thin water films. Microorganisms may be transported in the subsurface along with ground water movement, in the same manner as chemical solutes. Some microorganisms, however, are capable of motility, and are thus not necessarily restricted in their ability to move through fluids and across solid–water interfaces by strictly physical transport processes.

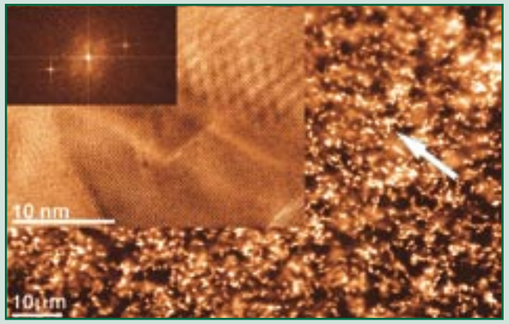

Figure 2.2. An epifluorescent photomicrograph of *Desulfovibrio desulfuricans* G20 attached to a hematite surface after 17 days into culture. The image is overlain with a high-resolution transmission electron micrograph of the resulting crystalline phases. The image in the upper left corner is a Fourier transformation of the 5.1 Angstrom lattice pattern indicating a hexagonal structure suggesting the mineral pyrrhotite. Arrow points to bacteria, which look white. (Image courtesy of B.M. Peyton, Washington State Univ.)

Most microbes in subsurface environments are attached to mineral particle surfaces. Thus, new models developed to predict geochemical reactions in the subsurface might include the ability to predict microscale changes in redox potential. Figure 2.2 shows sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) attached to a hematite (a type of Fe-(hydr)oxide) surface. Secondary minerals such as pyrrhotite (a crystalline Fe sulfide) can be formed on hematite surfaces as a result of microbial activity. Thus, microbes can influence the actual substratum on which they grow through their metabolic activity. Clearly, microbe–mineral interactions in the subsurface are dynamic and key to our understanding of this environment.

All microorganisms need carbon. Carbon can be provided in organic form (e.g., glucose or acetate), or in dissolved inorganic forms such as carbon dioxide. An existing contaminant can actually serve as a carbon source. This sometimes occurs with fuel spills. Agricultural wastes may be added as exogenous carbon sources, but carbon compounds produced in situ by plants or indigenous microorganisms may also help to support the desired microbial species. In some cases, inorganic nitrogen, sulfur, and/or phosphorus compounds are present in the environment in sufficient amounts to support microbial growth.

Bioaugmentation is the introduction of microorganisms that can biotransform or biodegrade a particular contaminant in a particular environment. Until recently, bioaugmentation had not been consistently effective in a subsurface environment as it was not clear whether the introduced species could be effectively distributed through the complex geologic structures of most subsurface environments or compete over the long term with the indigenous microbiota. However, recent studies show that *Dehalococcoides ethenogenes*, a small obligate anaerobe that can reductively dechlorinate tetrachloroethylene to ethylene, can be successfully introduced into the subsurface and might be useful in the cleanup of sites not previously adapted to anaerobic conditions for an extended time. Similarly, bioremediation can be enhanced by the continuous addition of microorganisms to a bioreactor for the above ground or ex situ treatment of contaminated groundwater. Organisms grown in the laboratory or produced in on-site bioreactors may also be added to ex situ treatments such as engineered soil piles, or injected back into the subsurface for in situ treatment.

Ex situ bioaugmentation is a technology commonly used at municipal wastewater treatment facilities. Commercial inoculants of enriched cultures consisting of one or more microbial species have been successfully used to colonize new trickling bed filter systems and rapidly recolonize systems where the intrinsic microbial community was victim to a system upset.

Researchers are beginning to investigate genetically engineered microorganisms (GEMs) for use in bioaugmentation. Genetic engineering is the manipulation of genes to enhance the metabolic capabilities of an organism. There is a great deal of interest in the in situ use of GEMs for the treatment of hazardous organic wastes. However, the application of genetic engineering technology for use in the environment remains controversial because of the concern that GEMs are not "natural" and may persist in the environment. The use of GEMs may be warranted when they are the only microorganisms that can transform or degrade a particularly hazardous contaminant.

Another potential future application of biostimulation and/or bioaugmentation would be in the formation of permeable barriers at the forefront of subsurface contaminant plumes. Contaminants might be immobilized within or near such a barrier by a combination of biological and geochemical processes, while the ground water would be allowed to pass through.

Permeable Reactive Barriers and Biobarriers

Permeable reactive barriers (PRB) are in situ treatment zones that are engineered down-gradient from a contaminant plume. As ground water passes through the treatment zone, contaminants are adsorbed, reduced and precipitated, biodegraded, biotransformed, or chemically degraded. Typically, PRBs are designed as trenches or funnel and gate-type systems; however, a series of closely spaced injection points can also be used. Originally, the reactive zones of PRBs were filled with zero valence iron. These iron

walls have proven to be quite effective for treating chlorinated solvents. More recently, PRBs have also been used to treat acid mine drainage through the addition of wood chips and various carbon sources, which sets up biologically active reducing zones that biotransform metals as they pass through the PRB.

PRBs are most effective when the plume is shallow, contained, and actively moving in a predictable direction. Various strategies using peat, different electron donors, wood chips, iron, and stable phosphate (apatite), in combination and in layers, could provide a cost-effective solution for biotransformation and biostabilization of metals and radionuclides. Iron PRBs have demonstrated that microbiological activity (e.g., sulfate-reducing bacteria) in the active zone can enhance biodegradation of some solvents and reduction of some metals. However, it also has been demonstrated that this same activity can greatly enhance the precipitation rates of all the metals in the ground water, coating the iron particles and decreasing reactive surfaces on the iron and decreasing the efficiency of the PRB. In addition, if the biostimulation is too great in the PRB, the increased biomass can make the PRB less permeable by blocking pore spaces with biofilms, turning the PRB into more of a biobarrier (rather than an enhanced treatment zone).

Biobarriers are an effective bioremediation strategy if the intention is to contain the contaminant plume. Another successful biobarrier strategy is the injection of ultramicrobacteria (<0.2 μm), formed by stressing bacteria so that they are more easily injected. This is followed by injection of nutrients that cause the ultramicrobacteria to return to their normal size and plug the pore structure so that ground water flow will be inhibited in that area. This strategy has been used by the oil industry to help contain fluid-loss zones in the deep subsurface and enhance oil recovery, but it has only recently been used to contain contaminants in the near-surface and needs more development.

Phytoremediation

Phytoremediation is the use of plants to remediate contaminated soils within the rhizosphere, which is the soil that surrounds and is influenced by plant roots and their associated microbial communities. Two forms of phytoremediation are applicable to the removal of toxic metals and radionuclides from the environment: phytoextraction and rhizofiltration. Phytoextraction is defined as the use of metal-accumulating plants to remove those contaminants from soil. Rhizofiltration is the use of plant roots to remove toxic metals and radionuclides from contaminated waters. Hyperaccumulation of heavy metals (greater than 1% of dry weight) is common for plants that are acclimated to soils with high concentrations of cobalt, copper, chromium, lead, nickel, and zinc. The plant root system serves both as a means for effective soil colonization by microorganisms and as a ready source of nutrients, with the result that microbial activity in the rhizosphere is greater and more easily sustained than in soils that lack a rhizosphere. Plants are also known to take up and transform organic and some inorganic compounds. Phytoremediation technology is relatively inexpensive compared to conventional technology and should prove cost effective for soils in which near-surface contamination is dispersed over broad areas.

Mycoremediation or Fungal Remediation

Mycoremediation, or remediation using fungi, is another approach that may be useful in the cleanup of contaminated soils and sediments. Fungi account for most of the biomass in soils, and they are known to have powerful biodegradative abilities. Fungi are also known to accumulate metals, particularly radionuclides (as observed following the 1986 nuclear reactor accident at Chernobyl in Ukraine). Further investigations are needed to determine these organisms' utility in decontaminating soils containing heavy metals. Some of the rhizofiltration activity ascribed to plants may be carried out by root-associated, mycorrhizal fungi. Mycoremediation could be as cost effective as phytoremediation, but would probably require the addition of fixed carbon. Most fungi require oxygen for growth, so mycoremediation would probably be most useful for treatment of near-surface soils.

Landfarming, Soil Piles, and Composting

Landfarming is the mixing of waste with surface soil over a tract of land. This technique has been used extensively to treat sludges from domestic sewage and industrial processes. The wastes are applied to soil surfaces as sludges or aqueous slurries, and the mixture is aerated through tilling. Optimal soil–water content is maintained and supplemental inorganic nutrients (nitrogen, phosphorus, and potassium) added to stimulate microbial growth. Supplemental microorganisms may also be added. Although landfarming has been an efficient and cost-effective means for treating a variety of wastes, adverse environmental effects sometimes have resulted, and this original landfarming method has been largely discontinued in the United States.

A modified form of landfarming has been adopted to comply with revised environmental regulations. This modified form consists of soil biopiles, or prepared beds, constructed above ground within contained treatment cells. This allows control of volatilization, leaching, and runoff. A vapor control system is constructed to ensure that volatile organic compounds (VOCs) are captured or destroyed. Current methods include adsorption to activated carbon for VOC disposal or destruction off-site.

Composting is a process applied to soil biopiles that controls and utilizes heat generated by aerobic microbial metabolism. The material being composted serves as a source of nutrients for the microbes. Bulking agents, such as wood chips or straw, are often added to enhance air movement through a pile. This self-contained system generates and retains heat, eventually raising the temperature of the compost pile. Composting has been used to biotransform explosives and propellants, through the amendment of sediment piles with manure or molasses to supply additional organic nutrients and microorganisms.

Land farming, prepared beds, biopiles, and composting hold a number of possibilities for bioremediation of radionuclides and metals by degrading organic chelating agents, altering pH, changing redox potentials, and producing biosurfactants. Any of these processes might be used to either mobilize, immobilize, or biotransform radionuclides and metals.

Slurries and Soil or Sediment Washing

Slurry bioreactors and soil- or sediment-washing equipment are commonly used to treat excavated soils or sediments to which water is added. Slurry bioreactors are stirred tanks within which biodegradation or biotransformation takes place in an aerated environment. Washing, which can be used in conjunction with the slurry process, is primarily a means of reducing the volume of contaminated soil or sediment by solubilizing readily desorbed contaminants and physically segregating the finer-grained portions of the sample to which contaminants tend to stick. The washing step can be performed with or without accompanying biological treatment. Excavated soils or sediments are screened to remove large debris, such as pipes, bricks, and concrete. Screened soils or sediments are further divided by size into readily treatable material, such as sand and fine gravel, and silt-sized and colloidal material known as fines. The fines can be stored as contaminant waste or biotreated in a slurry reactor. The solubilized contaminants may be biodegraded or biotransformed in the initial washing or, alternatively, the now-contaminated wash water can be passed to a second reactor where biological treatment takes place.

CONTAMINANT PLUMES:
MIGRATION OF HAZARDOUS WASTE IN THE SUBSURFACE

Contaminant plumes are zones of pollution extending downstream from sources of contamination. Contaminant types can vary in their rate of movement and distribution. If more than one contaminant type has been released into the subsurface, multiple plumes can form with different spatial and temporal distributions, and with different relative concentrations of contaminants. Although a contaminated site can have a number of plumes with different contaminants or contaminant combinations, this feature examines the characteristics of a single "composite" contaminant plume (Figure 2.3).

A source of contamination may be a single point such as a leaking tank. Point sources are frequently spills, treatment lagoons, and disposal sites such as trenches, landfills, and underground storage tanks. Or, the plume may have resulted from a "non-point source" of contamination of a large area, such as surface water that contains agricultural runoff contaminated by the general use of fertilizer on farmland.

Once a contaminant is released into the environment, the plume can spread into soils, unconsolidated sediments, rock formations, ground water, and surface water. The contaminant itself may be in gaseous, liquid, or solid form, or a combination. Depending on the geologic and hydrologic conditions at the site and the solubility of the contaminant, the plume may stay close to the source or be transported long distances by ground water or rainwater infiltration events.[2] In some cases, all of the contamination is caused by a single spill or leak. In others, the source of contamination may continue for decades, such as at an active waste disposal site or when natural infiltration by rainwater or other surface water percolates down through the zone of contamination.

In ground water, the shape of a plume will depend on the rate of migration, which is largely controlled by ground water flow, the hydrogeological setting, the physical and chemical characteristics of the contaminant, interactions between the contaminant and other dissolved substances, and the presence of a continuing contamination source. If the flow from the source has been stopped, the entire plume may migrate away from the original location, eventually becoming less concentrated through the transport processes of advection, diffusion, and dispersion, as well as by chemical and biological reactions. These factors are briefly described below.

Advection is the transport of dissolved solutes with the bulk flow of water. For highly soluble contaminants that do not undergo chemical or biological reactions with geologic materials, advection is the primary mechanism influencing the fate and migration of the contaminant. Dispersion is the mechanical mixing of solutes that occurs as the solutes are advected through the ground water system. Diffusion is the bulk movement of solutes resulting from thermally driven molecular motion of solutes. Through this random molecular motion, contaminants move from areas of high concentration to areas of lower concentration. Diffusion is thought to be particularly important when a geologic formation has a very low permeability or is very heterogeneous, such as a layered sequence of sand and clay.

[2] Hydrogeology and biogeochemistry in the vadose zone (unsaturated zone above the water table) are particularly important to DOE since some high-level radioactive waste (HLW) storage tanks in the vadose zone at DOE sites have leaked over the last 50+ years. The leaks have been sporadic, and the composition of the waste in the HLW tanks has changed over the years. The pH of the solution in the tanks (>12), the temperature (>90°C, due to radiolytic decay), the presence of complex organics, the presence of multiple radionuclides with different valences and solubilities, and pumping activities in the tank can have extreme effects on the mobility and transport of contaminants and the activity of microorganisms in the vadose zone. Thus, the waste and waste-site activities can influence the composition and concentration of contaminant plumes in the vadose zone.

Chemical and biological reactions can also affect the size and shape of the plume, primarily by slowing or accelerating migration of the contaminant. If the contaminants adsorb onto the geological materials, the rate of plume movement will be retarded (relative to the rate that water itself moves). Sometimes, however, contaminants adsorb onto very small particles, called colloids, which may themselves move with ground water flow, thereby transporting the contaminant.

In some cases, higher densities of microbes and higher concentrations of contaminants are observed at air–water interfaces, especially capillary fringe zones in the vadose zone immediately above the water table. Thus, water table fluxes can cause unexpected concentration phenomena at those interfaces. It is important to note that the bulk of subsurface microbial populations are associated with the solid phase. Chemical and biological interactions can result in precipitation of the contaminant into a solid phase that is no longer mobile. Organic contaminants can be degraded into simpler molecules — some of these may no longer be toxic, but in some cases the so-called daughter products may be more toxic. Radioactive contaminants will spontaneously decay into their daughter products, which will have their own set of transport properties and reactivities. These decay products may form solid-, liquid-, or vapor-phase contamination products of their own, which must be factored into any remediation strategy.

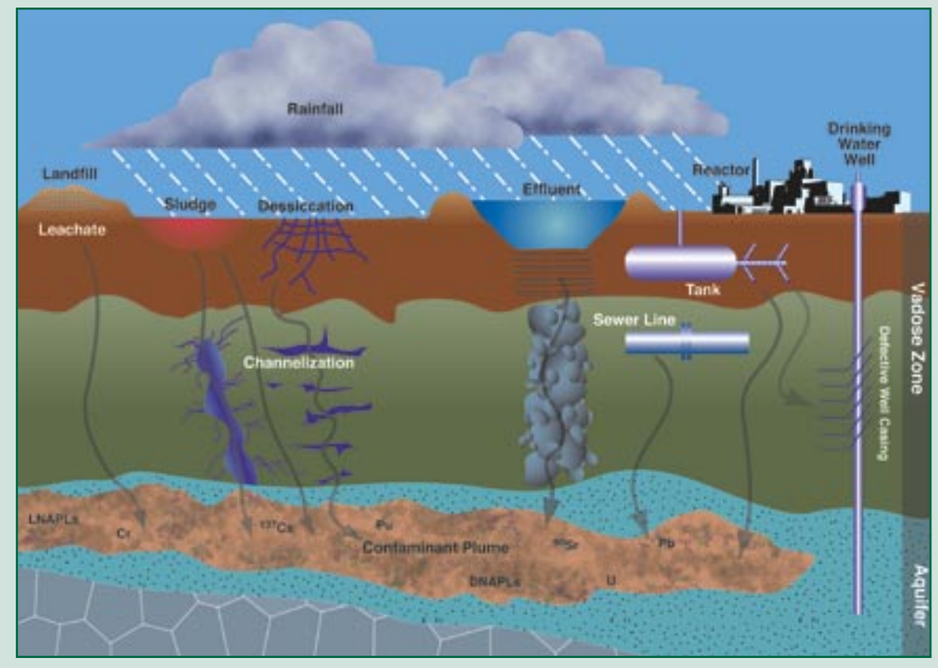

Figure 2.3. An example of a contaminant plume consisting of mixed waste resulting from percolation from leaky tanks, landfills, basins, and trenches.

SECTION III:

METALS & RADIONUCLIDES FOUND AT CONTAMINATED SITES

This primer looks at ways that microbial processes can be used to help remediate soils, sediments, and ground water contaminated with metals and radionuclides. Section II provided a general introduction to bioremediation and an overview of the various bioremediation technologies. This section describes some of the metals and radionuclides of most concern at many Department of Energy sites.

The contaminants of greatest interest are those that are long-lived and mobile, occur at a number of DOE facilities, and may pose risks to humans or the environment. These contaminants are the radionuclides cesium, plutonium, strontium, technetium, and uranium; and the metals chromium, lead, and mercury. Figure 3.1 illustrates their frequency of occurrence in ground water and in soils and sediments at DOE facilities. It is important to note that, of these radionuclides and metals, only uranium, technetium, chromium, mercury, and possibly plutonium, have been shown to be amenable to bioremediation as a cleanup strategy.

Metals and radionuclides are the source material for, and/or waste products of, nuclear fuel production, nuclear research, and nuclear reactor operations at DOE facilities. Many of the metals are also found in industrial and/or agricultural waste products. This section looks at how their transport properties and toxicity within the subsurface are influenced by their oxidation states, solubility, and adsorption. Transport and toxicity are both affected by contaminant form. One form or species of a metal or radionuclide may be harmless, while another may be toxic. In addition, one species may be mobile because it is water soluble, while another is immobile because it has precipitated or has been adsorbed onto a mineral surface.

RADIONUCLIDES

Radionuclides are physically unstable elements that decay spontaneously, emitting energy in the form of electromagnetic waves and/or particles. This natural process was discovered by the French physicist Henri Becquerel in 1895.[1] All elements, including hydrogen, have radioactive isotopes. The

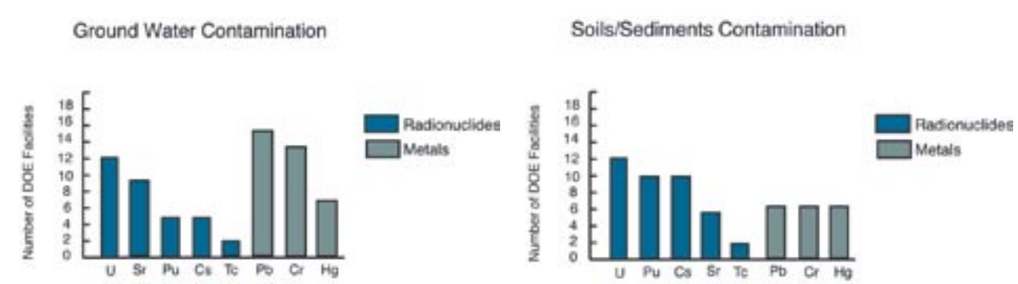

Figure 3.1. Frequency of occurrence of selected metals and radionuclides in ground water and soils/sediments at DOE facilities (adapted from Riley et al., 1992).

[1] Amounts of radioactive material are measured in units called becquerels, although exposures are described in terms of rems or rads — the amount of energy absorbed per unit mass.

OPPOSITES ATTRACT:
VALENCES, BONDS, AND REDOX REACTIONS

Atoms bond to achieve stability. Chemical bonds are formed by giving up, receiving, or sharing the electrons of the outermost region of an atom, known as the valence shell. These outermost electrons are the least tightly bound to the nucleus and are thus the most likely to participate in chemical reactions.

An atom's valence, or oxidation state, is the number of electrons an atom can give up or receive to achieve a bond. The oxidation state of an atom is indicated by a Roman numeral following the name of the element. Thus, iron(III), or Fe(III), indicates an iron atom in an oxidation state of +3. The uncombined Fe(III) ion is thus simply Fe^{3+}.

Two of the most important bond formations for bioremediation, particularly of metals, are ionic and covalent. In ionic bonds, a complete transfer of electrons from one atom to another occurs. This transfer creates two ions with an opposing electric charge, and is generally from a metal to a nonmetal. The metal loses one or more electrons and becomes a positive ion (a cation), and the nonmetal receives the electron(s) and becomes a negative ion (an anion). Electrostatic attraction between these ions of opposite charge bonds them to create a compound.

Ions must remain in association with ions of opposite charge. However, the requisite *counter-ion* may vary. Exchange of ions between different dissolved atoms (species), or between dissolved species and particulate matter that has a surface charge (e.g., silicate rock or organic colloids) is common. Ion exchange reactions are affected by pH, as both H^+ and OH^- may participate in this activity.

When atoms of two elements of about the same electronegativity react, they form covalent bonds. Covalent bonds result from the sharing of electrons such that each element involved has a filled outermost shell. This type of bond can form between near-neighbors in the periodic table, or between two atoms of the same element. Covalent bonds between identical atoms (such as H_2) are nonpolar, or electrically uniform, whereas those between unlike atoms are polar, with one atom being slightly negatively charged and the other being slightly positively charged. This partial ionic character of covalent bonds increases with the difference in the electronegativities of the two atoms. The distinction between ionic and covalent bonding is not absolute. Covalent bonds have a partially ionic character. Compounds often include both ionic and covalent bonds.

A coordinate association, or complex, is a special sort of covalent bond that was first described through the Lewis electron theory. Electrons can be exchanged between substances via the formation of these coordinate complexes. Certain molecules or ions can combine with others by forming a covalent bond with two electrons from a second molecule or ion. The first, or electron "acceptor," is called a Lewis acid, or electrophile. The second substance, which forms the covalent bond by "donating" a pair of electrons, is called a Lewis base, or nucleophile. Lewis acids and bases are thus conceptually very similar to traditional, or Bronsted–Lowry, acids and bases. However, it is specifically transition metals (those with *d*- and *f*-shell electrons, thus including most radionuclides), rather than other electropositive species, that tend to form coordinate complexes. All metal atoms or ions are Lewis acids. Most anions are Lewis bases. Coordinate complexes can form between metals and molecules of water, or between metals and ionic species such as carbonates or hydroxyl ions.

Oxygen is an important electronegative element in the study of environmental biogeochemistry. It has a valence of –2 in its ionic state, and thus requires the loss or sharing of two electrons to become stable. Within aqueous systems — including soils and sediments — oxygen may participate in ionic, covalent, and/or coordinate bonding. Most metals easily combine with oxygen to form metal oxides, and many ores consist of insoluble metal oxides. Thus, reaction with oxygen, or with hydroxyl ions, can affect the solubility of metals. Complexation of metals with the carbonyl ion (CO_3^{2-}) or other oxyanions (NO_x^-, SO_x^{2-}, PO_4^{3-}, ClO_4^-, etc.) can also affect solubility.

Electron transfer also plays a key role in environmental biogeochemistry. Oxidation–reduction, or "redox," involves a transfer of electrons between chemical species that may not necessarily result in bond formation. Ions or compounds that lose electrons are described as oxidized — even if oxygen atoms are not involved. Conversely, ions or compounds that gain electrons are described as reduced. For example, iron may be reduced to an oxidation state of Fe(II) (or Fe^{2+}) or oxidized to an oxidation state of Fe(III) (or Fe^{3+}). These differing oxidation states are sometimes referred to as chemical speciation.

Finally, elements can be oxidized or reduced within their natural environment. Air is, obviously, an oxidizing environment because of its 21 weight% free oxygen content. Subsurface environments may be oxidizing if the ground water present contains dissolved oxygen. The degree to which an environment is oxidizing is described as its redox potential, or Eh (also known as Eó). Valence determines reactivity (tendency to form bonds), and redox helps to determine valence. The role of microorganisms and redox in bioremediation will be explored in Section IV.

ratio of radioactive isotopes to all isotopes of a given element tends to increase with atomic number. Radioactive decay involves the emission of alpha particles, beta particles, or gamma rays. Gamma rays are the most energetic and therefore most likely to ionize whatever they strike; they can damage DNA, protein, and various human tissues. Radioactivity is not affected by the physical state or chemical combination of the element. Decay can be described in terms of half-life, or the time required for the activity (or the amount of the original radioactive material) to decrease by half. Half-life is not related to the energy released during decay. The radioactivity of a given material is most completely described by a combination of type (alpha, beta, gamma), energy, and half-life (Figure 3.2).

Alpha (α) emissions are particulate, and are described most simply as helium ions, in the form of He^{2+}, which has two protons and two neutrons, but no electrons. Their energies range from four to eight MeV (million electron-volts), and they do not penetrate more than a few centimeters through air. Beta (β) particles are identical to either electrons or positrons, depending on their charge. Their energies range from zero to four MeV, and they can be stopped by a thin sheet of metal. Gamma (γ) emissions are not particles, but are electromagnetic radiation of extremely short wavelength and intensely high energy (e.g., 1.25 MeV). They are very penetrating, and lead must be used to stop them. Radionuclides usually produce more than one type of emission during decay.

Of the naturally occurring radionuclides, only uranium and radium are found in substantial amounts. Most radionuclides are produced artificially in nuclear reactors or in particle accelerators. Others are produced during radioactive decay of uranium or other radionuclides. Radionuclides can be found as environmental contaminants at DOE sites as a result of the legacy of nuclear weapons production, testing, and research during the Cold War. Uranium and strontium have been reported in ground water at more than 50 percent of DOE facilities, and along with tritium are the most common radioactive constituents in DOE ground water. In soil and sediments at DOE sites, uranium, plutonium, and their decay products (such as strontium and cesium) have been cited as the most common radioactive waste components (Figure 3.1).

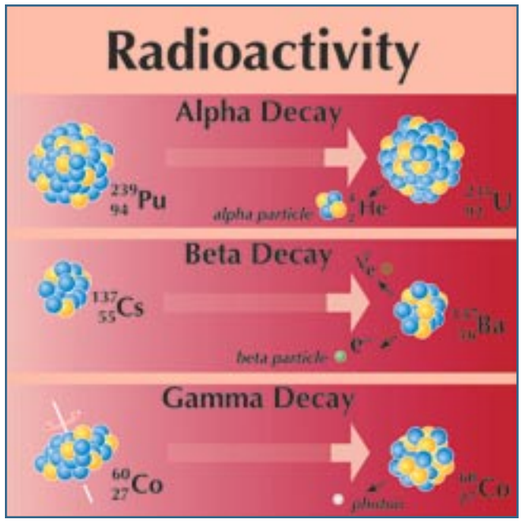

Figure 3.2. Radioactive decay transforms a nucleus by emitting different particles. In alpha decay, the nucleus releases a He nucleus—an alpha particle. In beta decay, the nucleus either emits an electron and antineutrino (or a positron and neutrino) or captures an atomic electron. Both alpha and beta decays change the original nucleus into a nucleus of a different chemical element. In gamma decay, the nucleus lowers its internal energy by emitting a high energy photon—a gamma ray. This decay does not modify the chemical properties of the atom. (Image adapted with permission from Contemporary Physics Education Project, http://www.cpepweb.org.)

Radionuclides in soils, sediments, and water can be present in many forms; that form is determined by the characteristics of the surrounding environment. Radionuclides form complexes with natural organic ligands such as humic substances. The solubility of these complexes varies with the pH of the natural aquifers in which they occur. Radionuclides also can form complexes with inorganic materials such as carbonate and sulfate. Natural organic matter (NOM) constitutes an important pool of ligands for complexing radionuclides and metal ions, and can play a role in their migration in subsurface environments. Some radionuclides are associated with colloids, which are microscopic particles suspended in a liquid medium, usually between 1 nanometer and 1 micrometer in size. In some cases, uranium, plutonium, and strontium at DOE sites were disposed with organic acids, complexing agents (such as EDTA), and solvents, all of which can influence the geochemical behavior and subsurface transport of radionuclides and metals.

Cesium (Cs)

Cesium is a relatively rare, silvery white metal, found in the Earth's crust. Cesium has only one naturally occurring isotope — 133. However, 20 radioactive isotopes have been created, with masses ranging from 123 to 144. The most hazardous and most frequently identified is cesium-137, which has a 30-year half-life.

Cesium-137 is a primary constituent of stored nuclear waste. Large quantities of cesium-137, along with strontium-90, were produced during the nuclear fuel cycle, specifically during the generation of plutonium and enrichment of uranium for use in nuclear weapon production. When the fissile weapons materials were then extracted from the fuel rods and processed as hazardous waste, the cesium and strontium were also extracted and processed, and the contaminants stored in waste storage tanks on DOE lands.

Cesium-137 and strontium-90 have been found in large quantities in fallout from the 1986 accident at Chernobyl, in the Ukraine. Because of cesium's similarity in chemical properties to potassium, cesium-137 is taken into the body in the same manner, and can result in whole-body radiation. In addition, the beta particles it emits are particularly toxic to bone marrow.

The cesium ion has only one oxidation state: +1. It gives up its electron very easily, forming ionic bonds with nearly all the inorganic and organic anions. Cesium easily loses electrons when struck by light, so it is used extensively in photoelectric cells and television cameras to form electronic images. The cesium-137 isotope is also useful in medical and industrial radiology. Cesium has not been shown to be transformed by microorganisms.

Plutonium (Pu)

Plutonium is a silvery metal that takes on a yellow tarnish in air. It is the second of the artificially produced transuranic elements. Very small amounts of Pu exist naturally. Synthetic Pu was first produced by deuteron bombardment of uranium-238. This event occurred in the U.S. in 1941, a year after neptunium (the first manmade element) was generated. Pu can be used for either electric power production (e.g., in satellites) or for nuclear weapons. Fifteen Pu isotopes exist, and all are radioactive. The half-lives of its isotopes (Pu-232 to Pu-246) range from 10^2 to 10^7 years. Its most important isotope is plutonium-239, which has a half-life of 24,100 years. Plutonium-239 is used for the production of nuclear fuel or nuclear weapons.

Plutonium generates alpha and gamma emissions during decay, and is known to mimic the behavior of iron (an essential element) in higher organisms. It is not absorbed through the skin (although release of highly energetic alpha rays causes localized tissue damage). However, Pu is extremely radiotoxic when absorbed via inhalation or ingestion (due to production of penetrating gamma rays). Plutonium exposure is linked to cancer of the lungs, liver, and skeleton. Its ability to catalyze the production of free radicals (from hydrogen peroxide or vitamin C) during radiolysis may induce oxidative stress, and hence cancer. It is unclear whether Pu is inherently chemically toxic; chemically analogous nonradioactive elements such as cerium and zirconium are fairly harmless. Plutonium's reputation as one of the most toxic substances known is probably related to its radioactivity. Permitted levels of exposure to plutonium are the lowest for any element.

Figure 3.3. Plutonium in +3 to +7 oxidation states in colored solution, from left to right: Pu(III), Pu(IV), Pu(V), Pu(VI), and Pu(VII).

Plutonium has five oxidation states (+3, +4, +5, +6, and +7; Figure 3.3). The solubility of Pu, like that of all elements, depends both on oxidation state and pH. The more insoluble form of plutonium is the Pu(IV) polymer, a hydrous plutonium oxide. However, in ground water the presence of complexing inorganic or organic species strongly influence the solubility of Pu(IV). For example, EDTA, a contaminant often found with actinide waste, is known to enhance solubility of Pu(IV), even in the polymer. Plutonium can also be present in ground water as a number of other compounds, including plutonium carbonates, plutonium hydroxides, and plutonium sulfates. In anoxic water, water-soluble plutonium occurs as the Pu(III) and Pu(IV) species, whereas in oxygenated waters, Pu(IV), Pu(V), and Pu(VI) may coexist. Plutonium(V) is known to predominate in seawater and oxygenated lake water. Plutonium is normally present in aerobic environments as the precipitate PuO_2(am).

The transport of Pu species depends on the oxidation state and the solution chemistry of the ground water (e.g., pH). Plutonium forms very strong complexes with a variety of organic ligands that affect its mobility in subsurface environments. These ligands include naturally occurring organic complexing agents such as humic and fulvic acids, microbially produced complexing ligands such as citric acid, as well as synthetic chelating agents.

Ethylenediaminetetraacetate (EDTA) is a synthetic chelator that can solubilize and transport radionuclides such as Pu in ground water. Understanding and predicting the form of PuEDTA in solution is critical to understanding the ground water transport properties, stability, and biodegradability of PuEDTA in the environment. Thermodynamic stability constants for the formation and dissociation of PuEDTA complexes are needed to predict the nature of soluble Pu species in different geologic environments. Recent published results have shown that the presence of EDTA significantly enhanced the solubility of Pu in aerobic environments over a range of pH values and raised Pu concentrations significantly above the drinking water limit of 10^{-12} M. Thermodynamic stability constants were developed for a variety of PuEDTA aqueous species and were used to accurately model the data. The dominant PuEDTA species at neutral pH is $Pu(OH)_2EDTA^{2-}$. This would be the dominant species present in solution at DOE ground water sites and available for biodegradation by EDTA-degrading bacteria.

Strontium (Sr)

Strontium was first found in strontianite ($SrCO_3$), a carbonate mineral. Its other natural ore is celestite

($SrSO_4$). It is an alkaloid metal with one oxidation state: +2. Chemically, strontium is similar to calcium and barium. The four naturally occurring isotopes are: Sr-88 (82.56%), Sr-86 (9.86%), Sr-87 (7.02%), and Sr-84 (0.56%). Approximately 16 artificial radioisotopes have been produced by nuclear reactions, of which the longest lived and best known is strontium-90 (with an approximate 28-year half-life). Along with cesium-137, strontium-90 is produced in large quantities during the fission process, so it exists in high concentrations in stored nuclear waste. It is also considered a potentially dangerous constituent of radioactive fallout (see cesium, above). Because it is chemically similar to calcium, strontium-90 can replace some of the calcium in foods and ultimately become concentrated in bones and teeth, where it continues ejecting ions that cause radiation injury. Although strontium is found in soils, sediments, and ground water at DOE sites, there is little evidence that microbes transform strontium. However, microbes may stimulate the precipitation of strontium as a $SrCO_3$ phase.

Technetium (Tc)

Technetium is unusual in that it is a relatively low atomic-number element, but is radioactive nonetheless. While primordial Tc has long since decayed on Earth, it is present extraterrestrially, such as in cool red stars. Technetium is a silvery and slightly magnetic metal that slowly tarnishes in air to a grayish powder. It was first synthesized in 1937, in Italy, within a sample of molybdenum that had been subjected to deuteron bombardment in the U.S. It is now found in the fission products of uranium and plutonium.

Twenty-five Tc isotopes exist (Tc-90 to Tc-108); all are radioactive. Only three Tc radioisotopes have half-lives of more than 102 years. The Tc of concern for environmental management purposes is technetium-99 (with a 212,000-year half-life),[2] which is produced in kilogram quantities as a fission product in nuclear reactors. It is derived from uranium and plutonium fission, and is used to absorb slow neutrons in reactors. Technetium releases gamma and beta rays during radioactive decay; the basis for its toxicity is primarily radiological.

Technetium can assume all oxidation states from +7 to 0. However, oxidation states +4, +5, +6, and +7 have the strongest potential to exist in the environment, with Tc(VII) and Tc(IV) dominating. The Tc(VII) pertechnetate ion (TcO_4^-) is highly stable in water under oxic conditions and may represent the species that is most mobile in ground waters under these conditions. Leaked technetium-99, described above, is believed to be in the form of TcO_4^-. Pertechnetate can be both mobile in ground water and bioavailable, and thus constitutes a significant part of the potential radioactive dose to humans at Tc-contaminated sites. Under anaerobic conditions, however, Tc(IV) can exist in the less soluble sulfide, carbonate, and/or oxide forms. However, if an anionic complex is formed, even reduced Tc can be as mobile as pertechnetate.

Technetium-99 may enter the environment via several avenues, such as through the separation and enrichment of uranium, and thus is present in stored wastes at a number of DOE sites, including Hanford, Paducah, Oak Ridge, and Portsmouth. These radionuclide wastes, originally stored in lagoons and burial pits, leaked into the subsurface and formed plumes in the sand aquifers below the vadose zone. The technetium-99 in these plumes is believed to be in the form of TcO_4^-.

Uranium (U)

Uranium, with an atomic number of 92, is the heaviest known natural element. It is a dense, hard, silvery-white metal that is both malleable and ductile. This metal tarnishes in air, and can actually ignite spontaneously. Uranium was discovered in 1789, in Germany, and was first isolated in 1841, in France. Uranium occurs in a number of minerals, including carnotite and uraninite, a dense black variety of which is called pitchblende. Uranium is not especially rare: deposits are found in the Democratic Republic of the Congo, eastern Germany, the former Soviet Union, France, Canada, South Africa, Australia, and the western United States. It is the 49th most abundant element in the Earth's crust.

Seventeen U isotopes exist (U-226 to U-242), and all are radioactive; their half-lives range from 10^5 to 10^9 years. In its natural state, uranium consists of a mixture of U-238 (99.27%), U-235 (0.72%), and U-234 (0.006%), with half-lives of 4.5 billion, 7.13 million, and 247,000 years, respectively. Uranium isotopes release alpha particles, beta particles, and gamma rays during their decay.

Uranium has four oxidation states: +3, +4, +5, and +6, but tends to exist in the U(VI) and U(IV) forms. Uranium(VI) is generally water-soluble and readily forms the uranyl ion [$(UO_2)^{2+}$]. (Uranium(VI) phosphates are an exception — uranyl phosphates are quite insoluble.) Uranium(IV) is generally water-insoluble and precipitates as uraninite [$(UO_2)^0$] or coffinite (a silicate mineral).

Uranium is used for nuclear research, nuclear fuel production, and nuclear weapons manufacture. Within the DOE complex, uranium manipulation has included mining, milling, refining, purification, enrichment, fabrication, and reprocessing. Therefore, uranium contamination is widespread. Uranium-235 is one of the two fissile materials used for the production of nuclear weapons, and in some nuclear reactors acts as a source of energy. Through the process of enrichment, a frequent source of uranium contamination, the amount of uranium-235 in natural uranium is increased for use in reactors. (A readily fissionable isotope, uranium-235 was used for the first atomic weapon.) Uranium-238 is used as feed material for the production of Pu-239, the other material used in the production of nuclear weapons (and used in the second atomic weapon). Plutonium-239 is virtually nonexistent in nature and is made by bombarding uranium-238 with neutrons in a nuclear reactor:

U-238 + neutron → U-239 → Np-239 → Pu-239

Uranium hexafluoride (UF_6), an interim product of the enrichment process, contains the soluble U(VI) ion and is highly radioactive and toxic.

Because of its importance in the fission process, large amounts of uranium are found in stored and discarded nuclear waste. In many cases, uranium is co-disposed with nitrate, because uranyl nitrate is generated by the leaching of uranium ore.

Uranium mining to obtain yellowcake is another major source of contamination by uranium decay products. Yellowcake is a yellow or brown uranium oxide powder that is processed to obtain uranium dioxide (UO_2) and uranium metal for use in reactors and nuclear weapons production. Conventional mining techniques generate a substantial amount of mill tailings, which may contain thorium-230 and radium-226. These isotopes have a half-life of about 75,000 years and 1,600 years, respectively. They can leach into ground water, and water samples near tailing piles have shown levels of some contaminants to be hundreds of times the government's acceptable level for drinking water. In addition, miners at these sites have died of lung cancers, which can be linked to inhaling uranium decay products.

The ionizing radiation from uranium (as well as other radioactive elements) can break chemical bonds, thereby destroying or damaging living cells. The most common routes of uranium contamination are through handling, ingesting, and inhaling. Inhaling and ingesting particulates contaminated with uranium can increase the risk of lung and bone cancer. Uranium is also chemically toxic at high concentrations and can cause damage to internal organs, particularly the kidneys. Uranium may also affect reproductive organs and the fetus, and may increase the risk of leukemia and cancers of the soft tissues.

Metals

Metals are commonly found contaminants in soils, sediments, and water, often in association with organic contaminants such as solvents and/or fuels. Metal pollutants can be inadvertently released during the manufacture of various industrial products, ranging from steel to computer components. Metal wastes can be produced through industrial processes such as mining, refining, and electroplating. Sludges and solid wastes can also contain metal contaminants. Some metals, such as elemental mercury, were used from the 1950s to 1970s in weapons production.

As with radionuclides, metals cannot be degraded, but they can be transformed through sorption, methylation, complexation, and/or changes in valence state. These transformations affect the mobility and

[2] Technetium-99m, not to be confused with technetium-99, has a short half-life of just over six hours. It is an important tracer radioisotope in nuclear medicine. Other Tc isotopes are useful as metallurgical tracers, as superconductors, or in the creation of high-strength magnetic fields at low temperature.

bioavailability of metals. At low concentrations, many metals are vital to life processes, often serving important functions in enzymes. However, above certain threshold concentrations, metals can become toxic to microorganisms and to higher species. Fortunately, microorganisms can alter the reactivity and mobility of metals, and thus facilitate the use of bioremediation as a form of treatment for metal-contaminated environments.

Chromium (Cr)

Chromium is a hard, brittle, semigray metal. It occurs in nature mainly as chromite ($FeCr_2O_4$), an ore mined in the former Soviet Union, Turkey, the Philippines, Zimbabwe, South Africa, and Cuba. Chromium is a transition metal, first isolated in 1780 in France, and is the 21st most abundant element in the Earth's crust. This element has been used since the beginning of the Industrial Age. Chromium has applications in nuclear, high-temperature, and metallurgical research, and as an alloying or plating material for corrosion resistance (e.g., for production of stainless steel). Chromium forms many complexes; indeed, "chromos" refers to the many colors of its various compounds.

The element is most often found in one of three oxidation states: +2, +3, or +6. However, a few stable compounds contain Cr in the +5, +4, or +1 state. The most commonly found oxidation states are +3 and +6, with +6 being the most toxic.

Chromium is an essential trace element that has a role in glucose and fat metabolism. Chromium deficiency leads to impaired glucose tolerance and elevated circulating insulin levels. However, too much Cr is known to cause skin irritation, or lung and kidney damage, indicating toxicity via all three toxicological routes (inhalation, ingestion, and skin contact), and also to cause cancer. Chromium can be hazardous as Cr(III) if inhaled. However, Cr(VI), which is water-soluble and therefore more bioavailable, is toxic and carcinogenic.

Chromium-bearing wastes are associated with reactor operations, fuel fabrication, and irradiated fuel processing at DOE facilities. The toxic and soluble Cr(VI) form is reported in soils and sediments on DOE lands (Figure 3.4). Chromium is not unique to DOE activities, however. Chromium(VI) can also enter the environment in effluents from metal plating operations and in industrial or municipal waste treatment plant discharges.

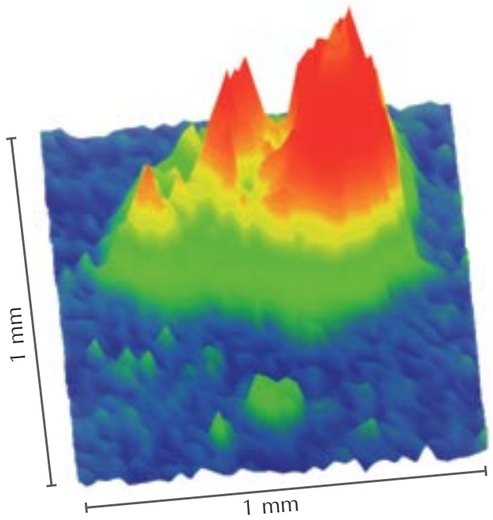

Figure 3.4. This computer image shows that chromium is present in highly localized chemical hot spots in the soil. The color scale ranges from blue, which indicates no Cr present, to red-orange, which indicates a concentration of one picogram per micrometer. The image was generated on the x-ray fluorescence microprobe beamline at Berkeley Lab's Advanced Light Source. (Image courtesy of Tetsu Tokunaga, Berkeley Lab.)

The highly soluble chromium(VI) can move through the subsurface environment. In ground water, it is typically found in the form of CrO_4^{2-} at neutral and high pH. Under acidic conditions, Cr(VI) occurs as $HCrO_4^-$. Chromium(VI) also sorbs onto Mn oxides and Fe oxides. Reduction of Cr(VI) to Cr(III) in sediments and ground water greatly reduces the solubility of chromium. Reduction of Cr(VI) can be caused by reaction with organic compounds (including natural organic matter), inorganic compounds (ferrous iron [Fe(II)], and sulfides), or through biotransformation by microorganisms.

Chromium precipitates occur primarily as Cr(III) compounds. Chromium(III) sorbs strongly onto Fe and Mn oxides, clays, and other mineral surfaces. Chromium(III) can be reoxidized to Cr(VI) through redox reactions with MnO_2. Biotransformation of Cr(VI) to the less toxic and less mobile Cr(III) presents an opportunity for bioremediation of chromium. However, the kinetics of the reductive reaction must be more rapid than those of the reoxidation reaction.

Lead (Pb)

Lead has two oxidation states, +2 and +4, and is toxic in both. It is bluish-white with a bright luster in its elemental state. Lead(IV) is generally the more soluble ion, but is far less prevalent in the environment. PbO_2 is a lead oxide that is soluble in water. Lead(II) is generally insoluble in ground water. Lead carbonate [$Pb(CO_3)$] and lead sulfate [$Pb(SO_4)$] are less soluble Pb(II) compounds. Lead(II) monoxide (PbO), in the forms of litharge and massicot, is also insoluble in water, but readily dissolves in acid. Lead in its insoluble form can be found with sulfur in the mineral galena (PbS) (Figure 3.5).

Lead wastes are associated with reactor operations, and lead ions are sometimes found in ground water at DOE facilities. Lead is also used extensively in industry. Great quantities of lead, both as the metal and as the dioxide, are used in storage batteries. Lead is used in ammunition and in radiation shields. Lead poisoning, also called plumbism, is caused by repeated exposure to the metal, resulting in its accumulation in the body tissues. Lead affects the intestines and central nervous system and causes anemia.

Children are especially susceptible to lead poisoning as the blood–brain barrier has not yet fully developed. Therefore, lead can more easily enter the brain. At lower levels of exposure (10 micrograms/deciliter), children can experience behavioral changes and decreases in intelligence. At high levels (150 micrograms/deciliter), children can suffer severe brain damage and die.

Environmental concern with lead poisoning has resulted in the elimination of lead from gasoline and paint products. Although elemental lead and some lead compounds are not absorbed by human tissue and are, therefore, not toxic, any soluble lead compound is toxic, with toxicity increasing as solubility increases.

Figure 3.5. Lead is usually found with sulfur in the mineral galena (PbS).

Mercury (Hg)

Mercury is rare in nature and is the only elemental metal that is liquid at room temperature. Its characteristically extremely high surface tension confers a unique flow behavior, and its linear thermal expansion and excellent electrical conductivity characteristics make it useful in many industrial applications. Liquid Hg is very dense, its specific gravity being six times that of water. In its solid form, Hg is silvery white, slowly tarnishing in alloys with most metals. Mercury's principal ore is the red sulfide, cinnabar (HgS). The ore is found in Spain, the former Yugoslavia, Mexico, Canada, and Algeria.

Mercury has been used since preindustrial times, sometimes with sad results — e.g., the expression "mad as a hatter" refers to the neurotoxicity associated with the use of Hg in the manufacture of hats (see toxicological information below). Mercury's more modern industrial uses include amalgams (such as those formerly used in dentistry), instruments (such as thermometers), and neutron absorbers in nuclear power plants.

Inorganic Hg exists in three oxidation states: 0 (elemental); +1 (mercurous); and +2 (mercuric). Mercury compounds contain either the Hg(I) or Hg(II) ion, although Hg(II) compounds predominate. All three oxidation states of inorganic Hg are toxic. The most volatile form, metallic Hg(0) vapor, is lipid soluble and readily absorbed via the respiratory tract. Less volatile, water-soluble methylmercury (CH_3Hg) is easily absorbed via the gastrointestinal tract, and also by inhalation. Water-soluble Hg(II) is modestly absorbed from the

gastrointestinal tract because of its low lipid solubility. In general, mercury is a cumulative toxin, with all forms tending to excrete very slowly once fixed in a tissue. Unfortunately, Hg is lipophilic. This perhaps explains the ease with which it crosses tissues after exposure via inhalation, ingestion, or skin contact, all three known toxicological routes. Mercury tends to concentrate at or within membranes, particularly those of the nervous system. It is a systemic toxin, as it disrupts calcium metabolism within all cells; absorption also leads to kidney damage. Because Hg associates with lipids, it tends to bioaccumulate in organisms and thus be transferred up the food chain.

Common anthropogenic sources of mercury include nuclear fuel production at DOE facilities as part of the uranium purification and isotope separation process (uranium-235 and uranium-238), industrial mining, burning of fossil fuels, and pesticides. Sewage treatment facilities are a widespread source of both inorganic and organic mercury compounds [Hg(0), Hg(II), methylmercuric chloride, and dimethylmercury]. Burning of fossil fuels contributes to atmospheric Hg.

The major form of Hg in the atmosphere is elemental mercury, Hg(0). Although it is the least reactive of the three oxidation states, Hg(0) is still poisonous because it is readily oxidized to the most reactive form, Hg(II), by both biotic and abiotic processes. This mercuric ion can then enter aquatic environments. Mercury in the form of Hg(II) also enters aquatic environments from industrial and nuclear fuel production wastes, and agricultural runoff waters. These pollutants then settle into river and lake sediments.

Ionic Hg(II) readily adsorbs to these sediments and other particulate matter. Microbial activity in aquatic and terrestrial environments can convert Hg to an organoelement via methylation. These forms of organomercury are highly toxic. For example, anaerobic sulfate-reducing bacteria, which commonly inhabit sediment, can methylate ionic mercury, forming methylmercury (CH_3Hg). Because it is both lipid and water soluble, methylmercury readily enters the aquatic food chain. Fish contaminated with methylmercury have been found in freshwater from Japan to the Great Lakes. Methylmercury is about a hundredfold more neurotoxic than ionic mercury [Hg(II)] and can be concentrated a millionfold in fish. Additional methylation by microorganisms produces dimethylmercury (CH_3HgCH_3), which is even more volatile and lipid soluble, but which must be partially demethylated before it can react with tissue proteins.

Fortunately, other microorganisms are known to demethylate organomercury. Although methylmercury is highly toxic, these bacteria have evolved genes that convert it to a much less toxic form. Thus, methylmercury is a suitable candidate for bioremediation. Demethylating microbes are often found in sediments containing the methylating sulfate-reducing bacteria. Their demethylating activities could be enhanced by several interventions, including but not limited to, amendment with native or non-native demethylating microbes or by phytoremediation. Alternative strategies, such as vapor extraction followed by collection of the volatile methylmercury, would require elaborate containment. This would be difficult for dry land decontamination or lake sediment remediation.

A LOOK AT MICROBIAL METABOLISM

Microorganisms are the most abundant life form on earth — both in the number of species and quantity and weight of living organisms. They have a history spanning over 3.5 billion years and have evolved to adapt to a wide range of environmental conditions and to survive on diverse sources of carbon and energy. Microorganisms are so named because they are usually too small to be seen with the naked eye (average size for an environmental microbe is about 1 µm or less). In a typical gram of soil, there are thousands of species of microorganisms and billions of individual organisms.

In soils and sediments, microbes play a key role in the degradation of plant-derived material, leading to the continuous cycling of carbon and nitrogen between the atmosphere and terrestrial biosphere. Microorganisms are also present at great depths below the land surface. Recent studies have shown that aquifers and oil reservoirs are inhabited by a diverse assortment of microorganisms that have learned to live in harsh conditions where temperatures and salinity may be high, and food (in the form of organic matter) is in short supply.

Microorganisms span the three domains of life: Eukarya, Bacteria, and Archaea. These three domains are divided according to the structure and biochemistry of their cells, including differences in their ribosomal RNA genes. Eukaryotic organisms have cells with a true nucleus. This domain includes higher multicellular organisms such as plants and animals as well as eukaryotic microorganisms, the ancestors of multicellular organisms. Eukaryotic microorganisms include algae, fungi, and protozoa. Bacteria and Archaea, however, do not have a discrete nucleus and are called, collectively, prokaryotes. Most prokaryotes are one-celled organisms, whereas eukaryotes may be one-celled or more complex, multicellular organisms. Archaea can be distinguished from Bacteria by the presence of isopranyl ether lipids in their cell membranes and the lack of the peptidoglycan in their cell walls.

Microorganisms can also be categorized according to their respiratory metabolic processes and sources of nutrition. This classification can be used to characterize their bioremediation potential. Some microorganisms, aerobes, require oxygen to grow, while others, anaerobes, are able to grow in environments devoid of available oxygen. Some organisms will grow on the simplest sources of carbon such as methane, while others will only grow on more complex carbon substrates such as cellulose. In sediment and ground water systems, there is a large diversity of organic molecules that can provide a source of carbon for microbial growth.

In addition to carbon, microorganisms also need electron donors and acceptors. Some metals and radionuclides can act as these donors and acceptors. Enzymatically catalyzed transfer of electrons (by oxidation and reduction reactions) between donors and acceptors releases energy for carrying out biochemical reactions. Microbial metabolism can play an important part in the transformation of metals and radionuclides, changing the form, or speciation, of these contaminants.

Bioremediation is a technology that uses metabolic processes to degrade or transform contaminants so they are no longer in a harmful form. In some cases, the contaminant is a primary part of the metabolic process, acting as the main source of carbon and energy for the cell. In other cases, the contaminant may be transformed while a second substance serves as a primary energy or carbon source. This "cometabolism" may be purely fortuitous, and the microorganism gains nothing from the process. Degradation of organic contaminants may result in daughter products that can be metabolized or in ones that persist.

Transformation of metals and radionuclides proceeds somewhat differently as they cannot be sources of carbon. However, metals and radionuclides can provide energy, and they can also be transformed indirectly in the energy transfer process. Metals and radionuclides can be transformed directly through changes in valence state by acting as electron donors or acceptors, or by acting as cofactors to enzymes. They can also be transformed indirectly by reducing and oxidizing agents produced by microorganisms that cause changes in pH or redox potential.

Transformation may also occur when microorganisms produce complexing agents that bind the metal or radionuclide, or degrade the complexing agent, or when the microorganism produces surfactants that desorb metals from sediments. The goal of this section is to introduce the reader to some of the basic metabolic processes involved in the biotransformation of metals and radionuclides.

Basic Microbial Metabolic Processes

Metabolism consists of the sequences of biochemical reactions, or pathways, in an organism that result in activity, growth, and reproduction. These include degradative (catabolic) and biosynthetic (anabolic) processes. Catabolic processes break down larger molecules into simpler components, producing energy for microbial growth and reproduction. Organic contaminants can be transformed into less harmful forms or degraded completely (mineralized) to inorganic components through these catabolic processes.

Some of the most important aspects of metabolism are: (1) the chemicals in the environment that serve as nutrient and energy sources; (2) enzymes, catalysts to the metabolic reactions that occur in the cell; and (3) oxidation–reduction reactions, which allow release and biological conservation of energy. Metals can serve important roles as electron donors or electron acceptors in these reactions.

Nutrient Sources

Carbon, nitrogen, and phosphorus are the basic elemental components of the most common molecules in a cell (proteins, sugars, and nucleic acids). Organisms that require an organic or complex source of carbon are called heterotrophs. Those that use inorganic sources of carbon like carbon dioxide (CO_2) are called autotrophs. Most microorganisms need nitrogen because it is a major constituent of proteins and nucleic acids. Nitrogen can be found in nature in both organic and inorganic forms. However, the most abundant forms of nitrogen in nature are inorganic — either ammonia (NH_3), nitrate (NO_3^-), or nitrogen gas (N_2). Most microbes can use either ammonia or nitrate as their sole nitrogen source. Nitrogen-fixing bacteria can use N_2 as a nitrogen source, fixing it directly from the air. Production of adenosine triphosphate (or ATP, the principal energy carrier molecule of the cell) and synthesis of nucleic acids and phospholipids require phosphorus, which occurs in nature in the form of organic and inorganic phosphates (PO_4^{3-}).

Microorganisms also need other nutrients, although to a lesser extent. The amino acids cysteine and methionine require sulfur. Most sulfur originates from inorganic sources, usually sulfate (SO_4^{2-}) or hydrogen sulfide (H_2S). Several enzymes need potassium, including some that are involved in protein synthesis. Potassium occurs in nature inorganically in the form of salts. Magnesium stabilizes ribosomes, cell membranes, and nucleic acids. Cells need iron in large amounts as it plays a major role in cellular respiration — it is a key component of the cytochromes and iron–sulfur proteins involved in electron transport. Most inorganic iron is highly insoluble, so many organisms produce specific iron-binding agents called siderophores, which solubilize iron salts and transport iron into the cell. Iron is found inorganically as Fe(III), Fe(II), and Fe(0) (elemental iron).

Energy Sources

Some microorganisms can use sources of energy other than organic compounds — light or inorganic chemicals. Those that use light are phototrophs, converting that light energy to chemical energy through photosynthesis; those that use chemicals are chemotrophs. Although many organisms obtain their energy from light, most microbes are chemotrophs. Microorganisms that use inorganic chemical compounds (such as metals and radionuclides) as a primary energy source are called chemolithotrophs.

Microbial Enzymes Acting as Catalysts

Enzymes are proteins that catalyze chemical reactions in the cell. Oxidation–reduction reactions are important in catabolic metabolism. These redox reactions transfer electrons and release energy from

Continued on Page 34

Who's Out There?
Microbial Species that Live in the Subsurface

One of the problems that has plagued scientists in bioremediation is how to identify and characterize the microbial communities that live in a contaminated site. Through laboratory culture techniques, microbiologists have been able to grow, at most, only one percent of the microbes in a community. Yet even when organisms can be cultured, they cannot always be identified.

Over the last few years, however, scientists have developed new ways of identifying microbes and assessing the microbial communities in the subsurface. The total microbial community can be examined, but no one method can furnish a complete analysis. Along with cultivation, however, each of these approaches provides a piece of the puzzle. Below are brief descriptions of some important tools for identifying and assessing microbial communities.

Culturing of Microorganisms on Growth Media. Culturing is a traditional method of identifying a microbial species (Figure 4.1). First a microbial strain representing a single species is isolated from a mixed culture and grown in a sterilized medium in a temperature-controlled incubator. Carbon sources, such as sugars and amino acids, may be added to the medium, as well as some kind of solidifying agent, such as agar. Researchers then perform a number of phenotypic tests to identify the cultured organisms by species. With bacteria, the first test will often be a Gram stain. This staining is based on a differentiation in cell-wall structure and chemical composition. Gram-negative organisms stain red and Gram-positive organisms stain purple. Then the microorganisms are put through further tests, the nature of which depends upon whether they are Gram positive or negative, until they are identified by process of elimination. Their fatty acid methyl ester (FAME) patterns and nucleic acid sequences can be used for further confirmation (see below).

Figure 4.1. Culture of Desulfovibrio vulgaris, a sulfate-reducer, showing FeS precipitation in media around colonies. (Image courtesy of T.C. Hazen, Lawrence Berkeley National Laboratory.)

16S rRNA Gene Sequencing. This identification method can be used with prokaryotes in the Bacteria and Archaea domains. It is based on determining the phylogenetic position of the unknown microbe among known microorganisms. This determination is based upon a particular gene in the cell's DNA (deoxyribonucleic acid) — that which encodes for 16S ribosomal RNA (ribonucleic acid). This molecule has excellent properties for making evolutionary comparisons. Its highly conserved sequences are an advantage in assessing major evolutionary divergences.

Obtaining the sequence of the DNA coding for 16S rRNA is accomplished in a variety of ways. One of the most common and effective uses a polymerase chain reaction (PCR)[1] to replicate ("amplify") the 16S rRNA gene. This amplified material is then sequenced.[2] Next, the sequenced 16S rRNA gene is compared to the sequences of other microorganisms that have been placed in a database of ribosomal rRNA genes (The Ribosomal Database Project at Michigan State University, http://rdp.cme.msu.edu). Microorganisms are classified according to the differences between the nucleotides in their 16S rRNA strands (Figure 4.2). Pairs of sequences from different organisms are aligned, and the differences in their nucleotide sequences are counted. The number of differences forms the basis for measuring the evolutionary distance between organisms. (See the inside back cover for a

[1] PCR is a technology that can enzymatically amplify minute quantities of specific gene fragments millions of times.

[2] For more information on how sequencing works, see the feature, "Genomics, Proteomics, and Bioremediation."

phylogenetic "tree" based on the Ribosome Database.) In addition, knowing the phylogenetic position of an unknown, uncultured organism may sometimes allow inference of its physiological properties, which in turn may suggest culture conditions that allow its isolation.

Analysis of Microbial Communities by Terminal Restriction Fragment Length Polymorphism (T-RFLP). Another approach that takes advantage of the use of ribosomal RNA for phylogenetic identification is terminal restriction fragment length polymorphism (T-RFLP) analysis. T-RFLP looks at the 16S rRNA molecule, but views only the terminal fragments generated from a restriction digestion of amplified community DNA. This approach rapidly screens communities for differences in structure. In addition, clone libraries may be constructed from microbial communities that have been impacted with contaminants such as Cr or U to identify surviving populations. (Clones are copies of DNA fragments that have been replicated by a phage or plasmid in a host bacterial cell.)

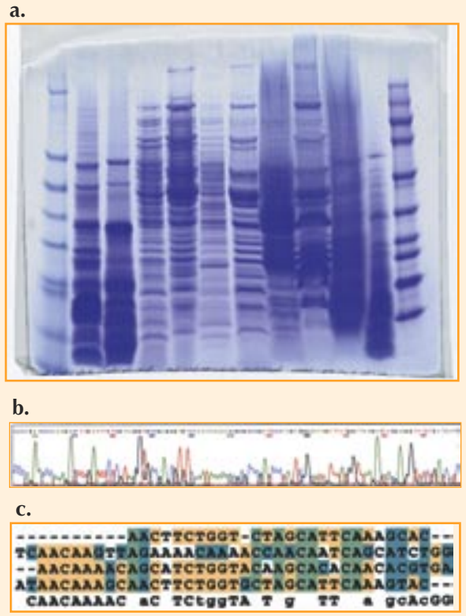

Figure 4.2. (a) RNA sequences are separated by gel electrophoresis. (b) Sequencing results are color coded by base type (adenine — green, guanine — black, cytosine — blue, and thymidine — red). (c) Alignment of four sequences, color coded to denote matching bases. (Images courtesy of Tamas Torok, Center for Environmental Biotechnology, Berkeley Lab.)

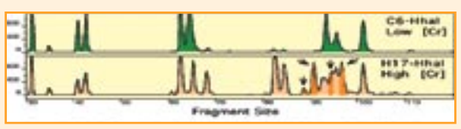

Figure 4.3. Terminal restriction fragment length polymorphism analysis of chromium impacted soils. Each peak is derived from the rRNA gene of at least one species. A comparison of soils with low and high concentrations of Cr identified populations that were unique to the Cr contaminated soils (indicated with arrows). These fragments were subsequently determined to be derived from members of the Cytophaga-Flexibacter group. (Image courtesy of T. Marsh, Michigan State University.)

At a chromium-impacted site, T-RFLP was utilized to correlate the Cytophaga-Flexibacter group of bacteria with high chromium content (Figure 4.3). Subsequent analysis of the community with a phylogenetically directed clone library allowed the identification of coherent phylogenetic clans within the Cytophaga-Flexibacter group that appeared to be selected for by the high chromium conditions. Analysis of isolates from a uranium-impacted site permitted the identification of a significant diversity of Gram-positive bacteria using this method. Identifying key microbial communities at a contaminated site will help to predict the effectiveness of intrinsic bioremediation and to optimize the growth of microbial communities involved in bioremediation.

Signature Lipid Analysis. This approach is based on extraction of the lipid[3] components of microbial cells. Extraction results in both a purification and concentration. Of the different lipids extracted, the charged polar phosphate-containing lipids provide insight into the extant community. All living cells are surrounded by a membrane formed of polar lipids. This is the water-resistant barrier between the outside world and the cell. The cells maintain this barrier by constant chemical activity, and when the cells die enzymes in the cells rapidly degrade these lipids so that they lose their charge. Consequently, the total polar lipids are a measure of the living cellular biomass. These polar lipids consist of a three-carbon alcohol glycerol with two fatty acids. The phosphate and other components occupy the third position.

The structures of these polar lipid fatty acids (PLFA) have a great deal of chemical complexity. Therefore, their patterns can be utilized, both in the identification of individual cultured isolates and for characterizing the total microbial community of a given environmental sample. Since most of the organisms in the total sample cannot be cultured, most of the organisms cannot be identified as to species. However, major classes of organisms can be quantitatively identified.

For example, Gram-positive organisms have a PLFA pattern that is much different than their red-staining, Gram-negative bacterial counterparts. Certain groups such as the actinomycetes, the Archaea, and the sulfate-reducing bacteria can be identified by their distinct patterns. Higher microbes, such as algae, protozoa, and fungi can also be identified. Physiological/nutritional status can be determined from shifts in specific lipid patterns induced in cultured organisms by stresses such as starvation, imbalance in nutrients, presence of sublethal toxicants, loss of oxygen, etc. Consequently, PLFA analysis provides the viable biomass, composition, and nutritional/physiological status of the community.

This information also allows investigators to find out not only *who* is out there but *what* the conditions are at a specific contaminant site. PLFA analysis "asks the microbes" if the various induced manipulations are effective. Investigators can then utilize shifts in the microbial ecology as a comprehensive and integrated monitor for toxicity assessment. Recently, signature lipid analysis was expanded by utilization of liquid chromatography/mass spectrometry. This adds much greater specificity and three orders of magnitude in sensitivity. With this technology it is now possible to detect microbes in one well (and at limits of only a few microbes) that were first injected into another well. This will be essential in manipulations involving bioaugmentation as a bioremediation strategy.

Fatty Acid Methyl Ester (FAME) Analysis. This approach is used to identify unknown bacteria through characterization of the fatty acid composition of the lipids in the microbial cell membrane. For the FAME analysis, microbial cell material is hydrolyzed, saponified in sodium hydroxide, then acidified with hydrogen chloride in methanol, causing the fatty acids to be methylated to form methyl esters. The fatty-acid-methylated esters are extracted with an organic solvent, and injected into a gas chromatograph. After the gas chromatogram

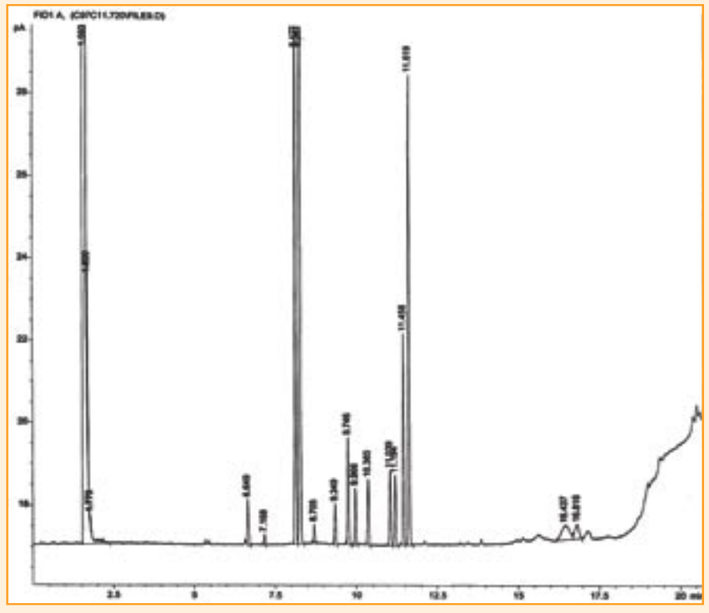

Figure 4.4. FAME chromatogram showing chromatographic column retention times and peak heights of a microorganism isolated from subsurface rock cores at Idaho National Engineering and Environmental Laboratory INEEL-10 test site. The 1.593 (far left) peak is the solvent peak. Remaining are carbon fatty acid peaks. All of these constitute a unique profile that can then be compared to those in the FAME database. This organism has a high similarity index to *Bacillus atrophaeus*. (Image courtesy of Tamas Torok, Center for Environmental Biotechnology, Berkeley Lab.)

[3] Lipids are the organic solvent-extractable, water-insoluble components of cells. These organic molecules are composed of fatty acids and a sugar molecule, usually glycerol.

profile of an isolate is obtained (with peak identification by mass spectrometry) (Figure 4.4), its FAME profile can be compared to those of known organisms in a FAME database using similarity indexes. The higher the similarity, the more likely the organism matches the database sample. There are only a few thousand species in this database, so identification is limited. However, the database is growing, and as new organisms are cultured their FAME patterns are added.

Artificial Neural Network Tools for the Analysis of Microbial Biomarker Data. A major challenge in the implementation of bioremediation technologies is understanding the composition of the indigenous microbial community and how this composition is affected by environmental conditions. Molecular biology provides new tools for characterizing the structure of these communities, and such techniques are especially useful when the community contains a large number of species that cannot be identified by traditional microbiological methods. However, these techniques can generate complicated data sets that are not easily analyzed by conventional statistical methods.

Artificial neural network (ANN) tools are being developed for analyzing microbial communities. An ANN analysis tool is capable of approximating continuous nonlinear functions using a computational paradigm that simulates the parallel and distributed processing mechanisms of the brain. An ANN may outperform a traditional linear model in predicting the complex relationships between environmental conditions and measures of microbial community structure.

For example, an ANN was "trained" to use geochemical measurements to predict the type and frequency of nitrite reductase (*nir*S) genes in ground water samples collected at the NABIR Field Research Center. The nonlinear ANN model explained most of the variation in the observed *nir*S types and frequencies (91.8%), whereas the linear model accounted for only 30% of the observed variation. This result suggests that there are patterns in the types of microorganisms containing the *nir*S genes that depend nonlinearly on the geochemistry. Understanding these relationships may be helpful in predicting the response of microbial communities under differing geochemical conditions.

Continued from Page 30

a substance. The substance that an enzyme acts upon is called the reactant or substrate. This is often the contaminant in bioremediation. A catabolic or anabolic pathway can contain a number of linked enzyme-catalyzed reactions.

For a reaction to occur, molecules must first reach a reactive state for chemical bonds to be broken. The amount of energy required to bring all molecules in a chemical reaction to the reactive state is called the activation energy. Once activation has occurred, the reaction can then proceed.

Catalysts are the substances that activate reactants. They do so by bringing reactants into a local chemical environment where conditions are favorable to proceed. Thus, the amount of activation energy needed to initiate a reaction is lowered. Catalysts also increase the rate at which a reaction will occur. However, they are not themselves changed by the reaction. Enzyme-catalyzed reactions occur very quickly. Enzymes can increase the rate of chemical reactions from 10^8 to 10^{20} times the spontaneous rate.

Some enzymes are highly specific in the reactions or groups of reactions they catalyze. In an enzyme-catalyzed reaction, the enzyme (E) temporarily combines with the reactant, or substrate (S), in an enzyme–substrate complex. The reaction occurs and the product (P) is released. This product is the transformed — oxidized or reduced — substrate. Then the enzyme returns to its original state:

$$E + S \leftrightarrow ES \leftrightarrow E + P$$

The interaction of enzyme and substrate usually depends on weak bonds to bind the enzyme to the substrate. To catalyze a reaction, an enzyme must bind the correct substrate, and position it correctly within the enzyme's active site. This places a strain on specific bonds in the substrate, which causes the substrate to break into component products. The result of this enzyme–substrate complex formation is a reduction in the amount of activation energy required to make the reaction occur and transform the substrate. Enzymes are often named for the substrate they bind or the chemical reaction they catalyze, denoted by "ase" at the end of the name. For example, ribonuclease is an enzyme that breaks down ribonucleic acid.

Oxidation–Reduction

Microorganisms obtain nutrients and energy for cellular processes and growth through oxidation–reduction reactions, which are catalyzed by specific enzymes. Oxidation–reduction, or redox, reactions involve the transfer of electrons from one reactant to another.[4] This transfer occurs through the donation of one or more electrons from a reactant serving as an energy source, called the electron donor, to another reactant called the electron acceptor. This transfer of electrons leads to changes in the chemical state of both donor and acceptor. In a redox reaction, the electron donor is oxidized and the electron acceptor is reduced. Because electrons cannot exist alone in solution, but only as parts of atoms or molecules, an oxidation cannot occur without a paired reduction.

In biochemistry, redox reactions often involve the transfer of not just electrons, but hydrogen atoms. When the electron is removed, the hydrogen atom becomes a proton (or positive hydrogen ion, H^+). In the oxidizing half-reaction $H_2 \rightarrow 2e^- + 2H^+$, the electron donor, hydrogen gas (H_2), is oxidized as it releases two electrons and two protons. In a second reducing half-reaction, the oxidation of H_2 can be coupled to the reduction of the electron acceptor O_2:

$$1/2O_2 + 2e^- + 2H^+ \rightarrow H_2O$$

The net oxidation–reduction reaction is balanced:

$$H_2 + 1/2O_2 \rightarrow H_2O$$

The tendency for a substance to donate or accept electrons is expressed by its reduction potential (Eh). Substances with large positive reduction potentials readily accept electrons. Substances with lower or negative reduction potentials readily give up electrons. Table 4.1 lists the reduction potentials for some of the most important redox half-reactions for microorganisms.

In soil and ground water systems with abundant carbon and nutrients for microbial activity, there is a sequence of redox reactions that typically occurs. First, nearly all of the O_2 is consumed by the reaction described above. When the O_2 is nearly depleted, nitrate (NO_3^-) is reduced to NO_2^-, NH_4^+, N_2O, and N_2 by reactions such as:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$$

$$NO_3^- + 10H^+ + 8e^- \rightarrow NH_4^+ + 3H_2O$$

Complete reduction of nitrate to N_2 is commonly referred to as denitrification. Manganese reduction, leading to the dissolution of solid-phase magnesium oxide, can begin while nitrate is present, by the reaction:

$$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$$

After nitrate is depleted, iron reduction leads to dissolution of Fe^{3+} minerals to aqueous Fe^{2+} by reactions such as:

$$FeOOH + 3H^+ + e^- \rightarrow Fe^{2+} + 2H_2O$$

$$Fe(OH)_3 + 3H^+ + e^- \rightarrow Fe^{2+} + 3H_2O$$

Finally, when the potential drops even lower, sulfate reduction becomes the predominant redox process, leading to the formation of reduced forms of sulfur such as HS^-, H_2S, and $S_2O_3^-$. Under even more highly reducing conditions, methane is generated by microbial reduction of CO_2 and organic carbon.

Because of the ubiquitous occurrence of these common earth elements, redox reactions involving contaminants must be viewed in light of where they lie in this redox sequence and how they compete or combine with these species for electron transfer reactions.

[4] See the feature "Opposites Attract: Valences, Bonds, and Redox Reactions" on page 20.

MICROBIAL RESPIRATION

Respiration is a fundamental metabolic process whereby microorganisms obtain the energy needed to grow and reproduce. There are two basic types of respiration: aerobic and anaerobic. Aerobic respiration occurs when oxygen serves as the terminal electron acceptor. Anaerobic respiration is the use of compounds other than O_2 as the terminal electron acceptor. Both types of respiration have been found to occur in soils, sediments, and aquatic environments. Mechanistically, the respiration in the presence and absence of oxygen has some similarities.

Aerobic Respiration

Aerobic respiration is very efficient because O_2 has a very positive redox potential, leading to a large difference in net reduction potentials between the primary electron donor and terminal electron acceptor. This means a greater release of energy and cellular conservation of ATP. Aerobic chemolithoautotrophs can use carbon dioxide as their sole carbon source, but also generate energy from inorganic compounds (electron donors) with oxygen as an electron acceptor. In aerobic respiration, compounds such as reduced iron (Fe^{2+}), ammonium sulfide $(NH_4)_2S$, or molecular hydrogen (H_2), can act as electron donors. These reactions hold promise for bioremediation because they can determine the fate and transport of radionuclides and other metals. For example, when dissolved Fe^{2+} is oxidized to Fe^{3+}, hydrous iron-oxide mineral precipitates are formed. These precipitates provide surfaces for reactions with other metals and radionuclides, allowing complexation to occur with contaminants, and thereby changing contaminant mobility.

Anaerobic Respiration

The reactions collectively known as anaerobic respiration are defined by their electron acceptor. The major modes of anaerobic respiration are denitrification, sulfate reduction, and ferric iron reduction. The processes of methanogenesis and fermentation may also be important in anaerobic environments. Some of the microorganisms that use these compounds as electron acceptors can also use metals and radionuclides (such as chromium and uranium) as terminal electron acceptors. However, because none of the electron acceptors used in anaerobic respiration have as large a reduction potential as the O_2/H_2O couple (Table 4.1), less energy is released when they are used. Thus, more substrate will need to be reduced to generate an equivalent amount of energy with redox pairs that have lower reduction potentials.

When inorganic compounds such as nitrate (NO_3^-), sulfate (SO_4^{2-}), and carbon dioxide (CO_2) are reduced for use by the cell as nutrient sources, they are said to be assimilated, and the reduction process is called *assimilative* metabolism. When these inorganic compounds are used only for energy metabolism as electron acceptors, this process is called *dissimilative* metabolism. In assimilative metabolism only enough of the compound is reduced to satisfy nutritional needs, and the reduced atoms are converted to cell material. In dissimilative metabolism, a relatively large amount of the electron acceptor is reduced to provide energy for the cell, and the reduced product is released into the environment. The focus of this section is on dissimilatory processes.

Nitrate Reduction (Denitrification). Basically, denitrification is the dissimilative reduction of nitrate (NO_3^-) to nitrogen gas (N_2), which the microbes couple to oxidation of a substrate to gain energy for growth. This is a two-step process. The first step is the reduction of NO_3^- to nitrite (NO_2^-). This is catalyzed by the enzyme nitrate reductase. The next step is the reduction of NO_2^- to N_2. This step is catalyzed by nitrite reductase and goes through the intermediates nitric oxide (NO) and nitrous oxide (N_2O). Dissimilatory reduction of nitrate to ammonia may also occur.

If oxygen is removed from a system and nitrate is present, denitrification will occur to the exclusion of most other forms of metabolism. Denitrification provides microbes with a relatively high amount of energy, and microbial growth yields are consequently high compared to other types of anaerobic metabolism. Under some conditions, the first step in the redox reaction (reduction of nitrate to nitrite) is faster than the second, and this disparity may cause the buildup of nitrite, which is inhibitory to many bacteria. Thus, denitrifiers may impact biological treatment of metals and radionuclides by inhibiting the activity of dissimilatory metal reduction or sulfate reduction, or by causing an increase in pH or depleting substrates. Denitrifiers can be integral to an in situ biological treatment approach if nitrate is one of the contaminants.

Table 4.1.
Microbially Significant Half-Reaction Reduction Potentials

Transformation	Reaction	Eh , Volts (@ pH 7)
O_2 depletion	$0.5O_2 + 2H^+ = H_2O$	0.82
Denitrification	$NO_3^- + 6H^+ + 5e^- = 0.5N_2 + 3H_2O$	0.71
Mn reduction, Mn(IV) to Mn(II)	$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	0.54
Fe reduction, Fe(III) to Fe(II)	$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	0.01
Sulfate reduction, S(VI) to S(–II)	$SO_4^{2-} + 10H^+ + 8e^- = H_2S + 4H_2O$	–0.22
Methane generation, C(IV) to C(–IV)	$HCO_3^- + 9H^+ + 8e^- = CH_4 + 3H_2O$	–0.26
H_2 generation, H(I) to H(0)	$H^+ + e^- = 0.5H_2$	–0.41

See Stumm and Morgan, 1996.

Most denitrifiers are facultative aerobes; that is, they can switch to denitrification when O_2 is no longer available as an electron acceptor. For example, some species of the genera *Pseudomonas*, *Bacillus* and *Thiobacillus* are capable of denitrification.

Iron Reduction. Iron is extremely abundant in the Earth's crust, primarily in the form of insoluble Fe(III) oxides. The reduction potential of Fe(III)/Fe(II) is electropositive (Table 4.1). A number of microorganisms are able to couple oxidation of hydrogen or organic compounds to the reduction of Fe(III) and gain energy for growth. The use of iron or other metals as terminal electron acceptors is called *dissimilatory metal reduction*. (Not all dissimilatory metal reduction, however, is linked to energy conservation.) Geological and microbiological evidence suggests that Fe(III) reduction was a very early form of respiration on Earth.

A phylogenetically diverse group of Bacteria and Archaea is known to conserve energy to support growth by oxidizing hydrogen or organic compounds (including contaminants such as aromatic hydrocarbons) with the reduction of Fe(III). Such a group includes species from such genera as *Geobacter, Desulfuromonas, Pelobacter, Shewanella, Ferrimonas, Geovibrio, Geothrix*, and others. These organisms have a broad spectrum of other metabolic capabilities as well. Many dissimilatory metal reducers such as *Geobacter* species can reduce soluble U(VI) to insoluble U(IV) (Figure 4.5).

Dissimilatory metal-reducing microorganisms might prevent migration of uranium in ground water by precipitation and immobilization in the subsurface. When a simple organic compound such as acetate is added to the subsurface, aerobic microorganisms quickly consume available dissolved oxygen and nitrate. Then dissimilatory metal-reducing microorganisms begin to metabolize acetate, oxidizing it to CO_2 while reducing available metals. While Fe(III) is generally the most abundant metal electron acceptor in the subsurface, dissimilatory metal-reducing

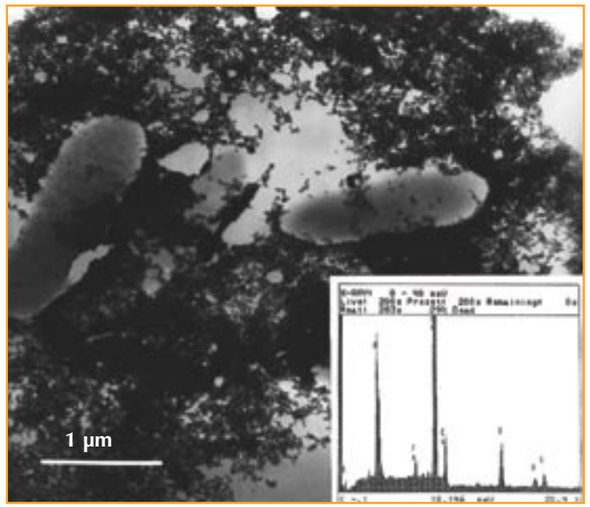

Figure 4.5. *Geobacter*, a microbe that can precipitate uranium, is commonly found in subsurface environments. (Image courtesy of D. Lovley, Univ. Mass.)

microorganisms can also simultaneously reduce U(VI) to U(IV), precipitating it out of ground water. This has been demonstrated conclusively in laboratory studies, and is the basis for new strategies of in situ bioremediation (see Section VI).

Mechanisms for iron reduction appear to vary among dissimilatory metal-reducing microorganisms (Figure 4.6). Some species use strategies to overcome the need for direct contact with Fe(III) oxides. For example, *Shewanella oneidensis* is a versatile microbe that can use oxygen, nitrate, uranium, manganese, and iron as electron acceptors. This bacterium appears to release quinones into the culture medium during growth that serve as electron shuttles between the bacterium and the Fe(III) oxide. *Shewanella alga* and *Geothrix fermentans* (in addition to producing electron shuttles) solubilize Fe(III) during growth, presumably by releasing one or more Fe(III)-complexing compounds called chelators. By contrast, *Geobacter metallireducens* does not release electron shuttles and is highly adapted to contact with the solid Fe(III) oxide. When growing on insoluble Fe(III) or Mn(IV) oxides, this microorganism produces flagella and uses chemotaxis to find the electron acceptor. Pili are also produced under these conditions, presumably to attach to insoluble oxides.

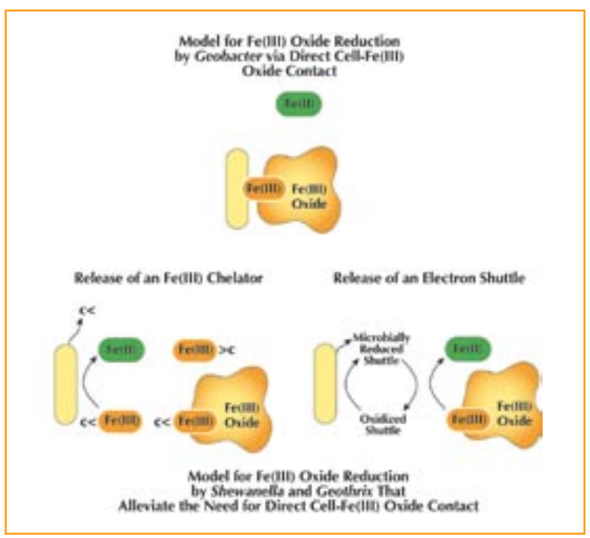

Figure 4.6. Mechanisms of Fe(III) reduction by dissimilatory metal-reducing bacteria (c= chelator). (Image courtesy of D. Lovley and *ASM News*.)

Although dissimilatory metal reducers are of obvious importance to developing strategies for bioremediation of organic contaminants as well as metals and radionuclides, this process can be slow. One idea for stimulating their activity in aquifer sediments is to add humic acids or other quinone-containing compounds to which Fe(III)-reducing microbes can transfer electrons. Electron shuttling via extracellular quinones may accelerate the rate and extent of bioremediation.

Sulfate Reduction. Sulfate (SO_4^{2-}) is used as an electron acceptor in dissimilative sulfate reduction. Sulfate reduction produces much less energy, however, than O_2 or NO_3^- (Table 4.1), and growth yields of microorganisms are lower. The first product of sulfate reduction is sulfite (SO_3^{2-}). The end product is hydrogen sulfide (H_2S). Most sulfate-reducing organisms are chemoorganotrophs, using various organic compounds as electron donors, including the fermentation (see below) products lactate, acetate, and ethanol. However, in some cases hydrogen gas (H_2) can be an inorganic electron donor. Sulfate-reducing microbes that grow using H_2 as an electron donor are chemolithotrophs.

The metabolic activity of sulfate reducers is not limited to the reduction of sulfate; other metals and nitrate may be reduced by some of these organisms. Furthermore, sulfate reduction and the direct reduction of iron can occur simultaneously, depending on how available the iron is to microbial reduction. *Desulfovibrio desulfuricans* is a well-known sulfate-reducing bacterium that can also use iron, uranium, or chromium as an electron acceptor.

Methanogenesis. Methanogenesis is the microbial production of methane (CH_4) through the reduction of CO_2 (Table 4.1). Carbon-dioxide reduction is coupled to oxidation of hydrogen, with hydrogen gas (H_2) being one of the most common electron donors. Organic compounds such as acetate, formate, and trimethylamine can also be electron donors. Methanogens are Archaea. These microorganisms are present in most anaerobic environments, including waterlogged sediments, marshes, rice paddies, and the gastrointestinal tracts of some animals. Although these reactions probably do not directly impact metals or radionuclides, they may have an indirect and possibly adverse effect by competing for substrates with dissimilatory iron reducers or sulfate reducers (which can catalyze reactions that affect inorganic contaminants). However, under many conditions relevant to in situ treatment of metals and radionuclides, the dissimilatory iron-reducing and sulfate-reducing microorganisms can successfully out-compete methanogens for the substrates.

Fermentation. Fermentation is an anaerobic process in which energy generation occurs by redox reaction and in which an organic substrate serves as both electron donor and electron acceptor. The organic compound, such as a sugar or amino acid, is broken down into smaller organic molecules, which accept the electrons that were released during the breakdown of the energy source. Although metals and radionuclides are not directly affected by fermentation, it can be an important step in the production of substrates used by dissimilatory iron-reducing and sulfate-reducing bacteria, which are the primary catalysts of reactions that affect inorganic contaminants. In addition, there is evidence in sediments that fermentation products can serve as metal complexing agents, increasing metal contaminant mobility.

EXPLORING THE DIVERSITY OF IRON(III)-REDUCING BACTERIA IN SUBSURFACE SEDIMENTS

Iron(III)-reducing bacteria are thought to catalyze a large number of sedimentary processes that have important impacts on bioremediation. Many new strains of iron-reducing microorganisms have now been isolated from uranium-contaminated subsurface sediments, expanding our knowledge of the diversity of this environment. Gene-sequencing methods have been used to classify these isolates, which include Gram-positive genera (*Clostridium*, *Carnococcus)* that were not closely related to any previously characterized pure cultures of Fe(III)-reducing bacteria.

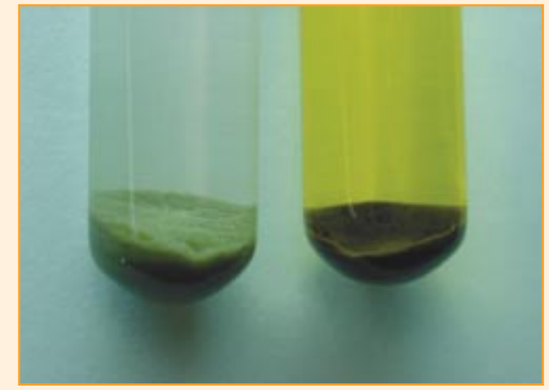

Figure 4.7. Iron-reducing *Clostridium* isolated from a uranium contaminated sediment. On the left is a culture of *Clostridium* in which U(VI) has been completely reduced with malate as the carbon source. On the right is a heat-killed control culture in which U(VI) was not reduced, as shown by the yellow color in the medium. (Image courtesy of J. Kostka, Florida State University.)

Most previously described Fe(III)-reducing bacteria (FeRB) have one cell membrane type (Gram-negative) and are classified within one group of Bacteria (the Proteobacteria Phylum). Yet, a few organisms with different cell membrane types (termed Gram-positive) and even prokaryotes of a different domain altogether (Archaea) have been shown to mediate this environmentally important process. Analysis of lipid biomarkers of these microbes revealed relatively high proportions of plasmalogens, a characteristic diagnostic of the *Clostridia*.

The *Clostridia* (Figure 4.7) are well-studied anaerobic bacteria that have been isolated from sediments since the origin of environmental microbiology. However, all of the *Clostridia* isolated previously were fermentative organisms incapable of respiration. In contrast, many of the *Clostridium* strains described from contaminated subsurface sediments were shown to conserve energy for growth by coupling the respiration of Fe(III) oxide minerals to the oxidation of organic acids (acetate or lactate). Several of the bacterial isolates were also shown to reduce U(VI). Although their environmental significance remains to be explored, these newly isolated FeRBs could play an important role in subsurface bioremediation.

Genomics, Proteomics, and Bioremediation

All living things, including microorganisms, have a chemical called DNA (deoxyribonucleic acid) that contains information used by the organism to build and maintain cell biomass, and to reproduce itself. The DNA molecule is made up of four chemical building blocks (bases): adenosine (A), thymidine (T), cytosine (C), and guanine (G). In microorganisms, millions of these bases form long strands that pair together (A with T, and C with G) in a twisted zipper-like structure known as a "double helix."

Genomics is the study of the complete set of genetic information — all the DNA in an organism. This is known as its genome. Genomes range in size: the smallest known bacterial genome contains about 600,000 base pairs and the human genome has some 3 billion. (The size of a genome is designated in millions of base pairs or megabases, abbreviated Mb.) Typically, genes are segments of DNA that contain instructions on how to make the proteins that code for structural and catalytic functions. Combinations of genes, often interacting with environmental factors, ultimately determine the physical characteristics of an organism.

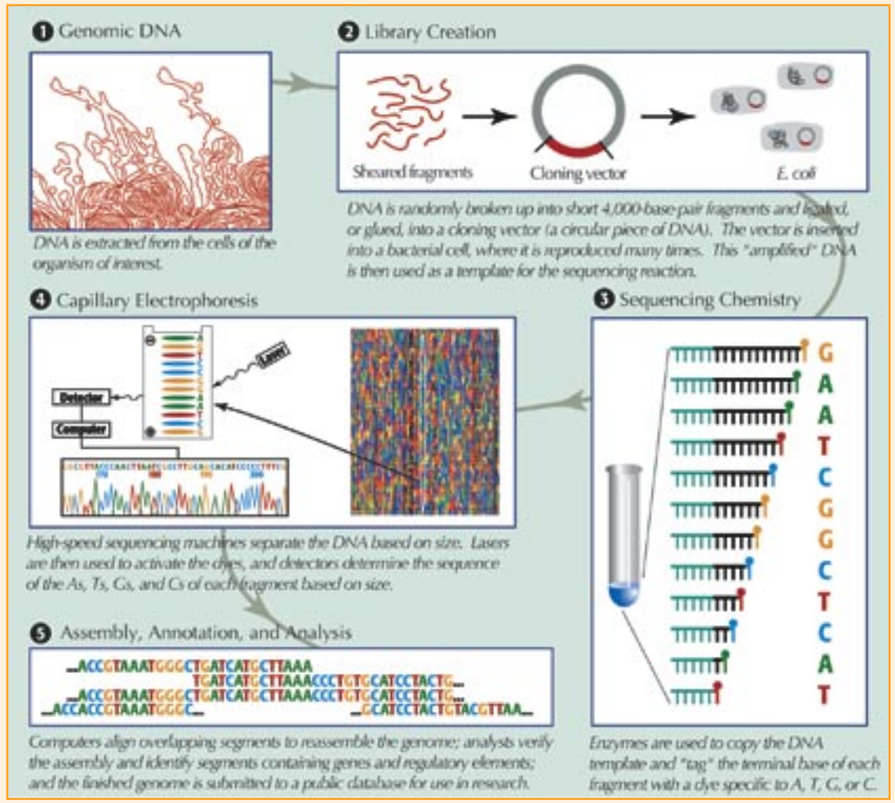

Figure 4.8. Sequencing genomes. While the number of chromosomes, genes, and base pairs in the genomes of different organisms vary, their fundamental structures are very similar, and the techniques for sequencing and studying them are the same. Whole genome shotgun sequencing is used to determine the order of the bases of an entire genome. (Graphic courtesy of DOE Joint Genome Institute, Walnut Creek, CA.)

How are genomes sequenced? At the DOE Joint Genome Institute and other sequencing centers, high-throughput procedures enable rapid sequencing of microorganisms (Figure 4.8). Microbial genomes are first broken into shorter pieces. Each short piece is used as a template to generate a set of fragments that differ in length from each other by a single base. The last base is labeled with a fluorescent dye specific to each of the four base types. The fragments in a set are separated by gel electrophoresis. The final base at the end of each fragment is identified using laser induced fluorescence, which discriminates among the different labeled bases. This process recreates the original sequence of bases (A, T, C, and G) for each short piece generated in the first step.

Automated sequencers analyze the resulting electropherograms, and the output is a four-color chromatogram showing peaks that represent each of the four DNA bases. After the bases are "read," computers are used to assemble the short sequences (in blocks of about 500 or more bases each, called the read length) into long continuous stretches that are analyzed for errors, gene-coding regions, and other characteristics. To generate a high-quality sequence, additional sequencing is needed to close gaps, reduce ambiguities, and allow for only a single error every 10,000 bases. By the end of the process, the entire genome will have been sequenced the equivalent of 8 or 9 times. The finished sequence is submitted to major public sequence databases, such as GenBank (http://www.ncbi.nlm.nih.gov/).

Once the genome has been sequenced, portions that define features of biological importance must be identified and annotated. When the newly identified gene has a close relative already in a DNA database, gene finding is relatively straightforward. The genes tend to be simple, uninterrupted open reading frames (ORFs) that can be translated and compared with the database. However, the discovery of new genes without close relatives is more problematic. Scientists in the new discipline of bioinformatics are developing and applying computational tools and algorithms to help identify the functions of these previously unidentified genes. An accurate accounting and description of genes in microbial genomes is essential to describing metabolic pathways and other aspects of whole-organism function.

The new scientific discipline of genomics is providing insights into some key microorganisms involved in metal and radionuclide bioremediation. They include *Geobacter sulfurreducens*, *Deinococcus radiodurans,* and *Shewanella oneidensis*.

Geobacter sulfurreducens (3.7 Mb) is a representative of the family *Geobacteraceae*, which is of major importance in subsurface environments (Figure 4.9). *Geobacteraceae* is the dominant group of Fe(III)-reducing microorganisms recovered from a wide variety of aquifer and subsurface environments when both molecular and traditional culturing techniques are used. *Geobacteraceae* are capable of oxidizing organic compounds, including aromatic hydrocarbons, to carbon dioxide with Fe(III) as the electron acceptor. *Geobacteraceae* can also reduce other metals such as Mn(IV), U(VI), Tc(VII), Co(III), Cr(VI), and Au(III). Therefore, *Geobacter* species may play a critical role in the remediation of contaminated anaerobic subsurface environments. Genomic sequencing has revealed many new aspects of *Geobacter* physiology and ecology. For example, genes coding for flagella found in the genome prompted additional studies revealing that the organism is chemotaxic toward iron, which may help it localize Fe(III) oxides. Moreover, the genome of *G. sulfurreducens* has coding regions for over 100 c-type cytochromes; these proteins may function in metal-reduction pathways.

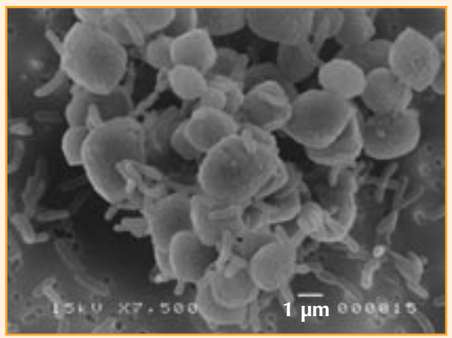

Figure 4.9. Geobacter sulfurreducens growing with insoluble Mn(IV) oxides as the electron acceptor. (Image courtesy of D. Lovley, Univ. Massachusetts.)

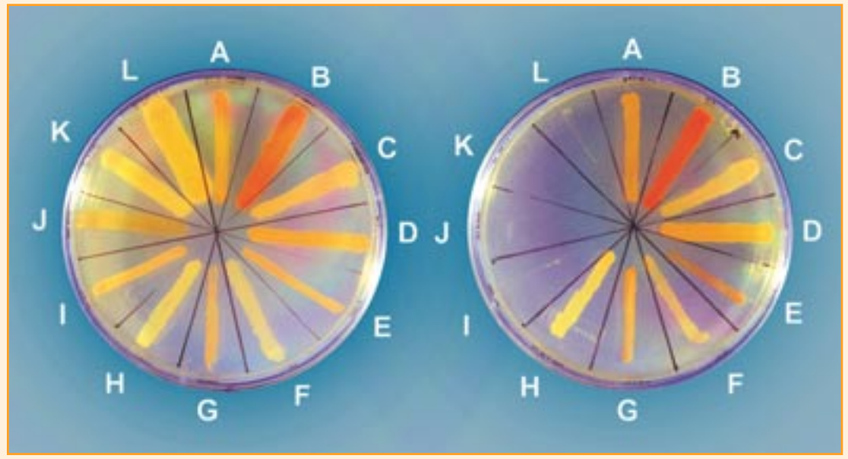

Figure 4.10. Growth of bacteria (family *Deinococcaceae*) in the presence of chronic irradiation (60 Gy/hr). Left, control plate incubated in the absence of radiation. Right, plate incubated in the presence of 60 Gy/hr gamma radiation (^{137}Cs). **A.** *Deinococcus radiodurans*; **B.** *Deinococcus radiopugnans*; **C.** *Deinococcus grandis*; **D.** *Deinococcus proteolyticus*; **E.** *Deinococcus murrayi*; **F.** *Deinococcus geothermalis*; **G.** *Deinococcus radiophilus*; **H.** Novel unclassified deinococcal species isolated from an elephant's trunk, National Zoo, Washington, D.C.; **I.** D. *radiodurans* rec30 (recA⁻), a mutant lacking a protective gene; **J.** *Shewanella oneidensis* (MR-1); **K.** *Escherichia coli*; **L.** *Pseudomonas putida* F1. (Figure courtesy of M. Daly, USUHS.)

Deinococcus radiodurans (3.2 Mb) survives extremely high levels of radiation (150,000 Grays; a dose of 500 is fatal to humans) and possesses an unusual ability to repair the resulting damage to its DNA (Figure 4.10). The complete genome sequence revealed the presence of two chromosomes (2.6 and 0.4 Mb) as well as a mega plasmid (177,466 base pairs), and a small plasmid (45,704 base pairs). Multiple components distributed on the chromosomes and megaplasmid contribute to the ability of *D. radiodurans* to survive under conditions of starvation, oxidative stress, and high amounts of DNA damage.

It has been demonstrated that *D. radiodurans* is naturally able to reduce/detoxify Cr(VI), U(VI), and Tc(VII). *D. radiodurans* has also been genetically engineered for remediation of Hg(II) and the fuel hydrocarbon toluene. By inserting genes from *Pseudomonas putida, D. radiodurans* is able to assimilate carbon, and use energy derived from toluene catabolism for growth and metal reduction. Thus, engineered *D. radiodurans* is a promising candidate for bioremediation of high-level radioactive wastes as well as mixed radioactive wastes containing both organic and metallic components. A dividing cell of *D. radiodurans* is shown in Figure 4.11.

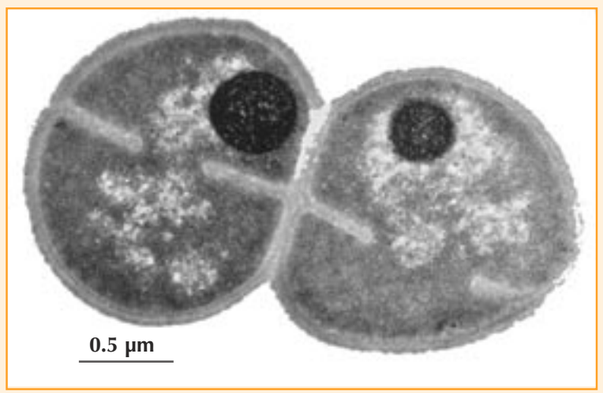

Figure 4.11. *Deinococcus radiodurans*, magnified 60,000 times. (Image taken by John Battista and Peggy O'Cain of Louisiana State University.)

***Shewanella oneidensis* MR-1** (4.5 Mb) can grow aerobically or anaerobically, utilizing an amazing diversity of electron acceptors, including nitrite, nitrate, thiosulfate, iron, manganese, and uranium. *Shewanella* can enzymatically reduce radionuclides and metals such as uranium, technetium, and chromium, transforming them from soluble form into precipitates. Figure 4.12 shows an environmental scanning electron micrograph of *S. oneidensis* on the surface of manganite, a manganese oxide, which can serve as an electron acceptor.

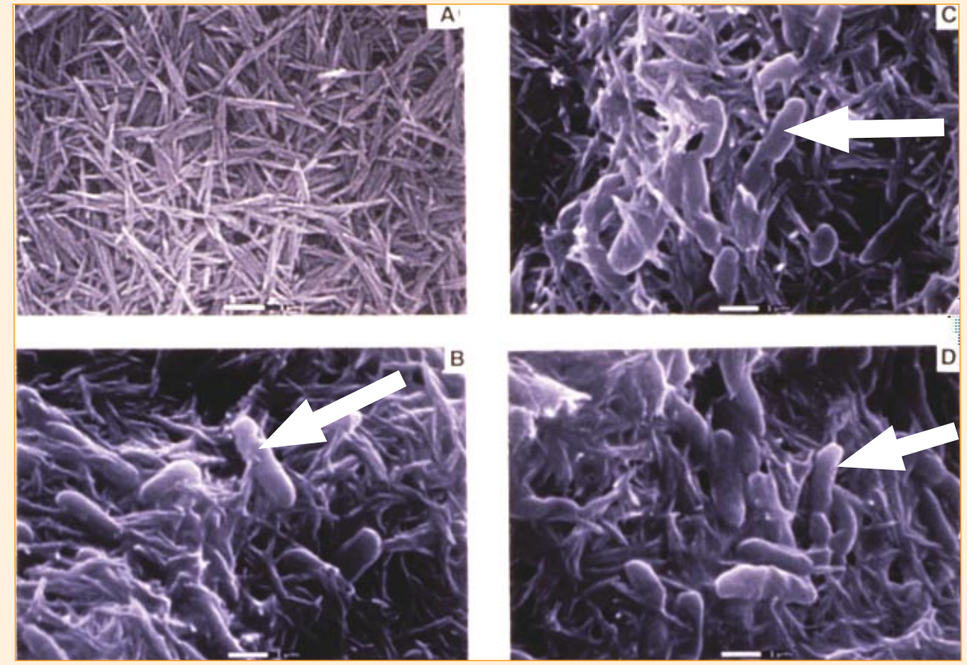

Figure 4.12. Environmental scanning electron micrograph of *Shewanella oneidensis* MR-1, a dissimilatory metal-reducing microbe, on the surface of a manganite, a Mn oxide. Panel A shows the metal with no bacteria, while B–D show the metal surface after a few days', microbial growth. (Arrow points to microbe on metal; bar = 1 µm.) (Image courtesy of K. Nealson, Univ. So. Calif.)

Proteomics is a new branch of science dedicated to studying all the proteins expressed by a cell and how these proteins change under different growth conditions. For example, scientists are trying to understand the protein expression pattern of the organism *Shewanella oneidensis*, whose respiration can be coupled to the reduction of U(VI) to U(IV). The proteins mainly responsible for this conversion are located on the outer membrane of the cell. Protein expression can be measured by isolating the proteins from cells and determining their relative abundance using protein separation and detection methods such as two-dimensional gel electrophoresis.

Mass-spectrometry-based techniques are also being used to identify the full complement of cellular proteins, with the ultimate goal of determining how the cell localizes different proteins on the cell surface when conditions change from aerobic to anaerobic respiration. The entire complement of proteins associated with bacterial outer membrane vesicles (MVs) has been determined by a new technique that involves the use of both high-resolution separation and high-mass accuracy and sensitivity Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry. MVs are unique to Gram-negative bacteria and are constantly being released from the cell surface during bacterial growth. During their release, MVs trap some of the underlying periplasm that contains various enzymes. MVs are thought to be how bacteria protect enzymes that are secreted extracellularly, and also the method by

which they deliver lethal enzymes into other bacteria as a means of predation, and even to eukaryotic cells early in pathogenesis. Identifying the entire protein complement of MVs can lead to predictions of their impact on the environments in which they are found. Results of whole proteome analyses suggest that outer MVs shed from *Shewanella oneidensis* MR-1 contain enzymes responsible for reducing metals and radionuclides.

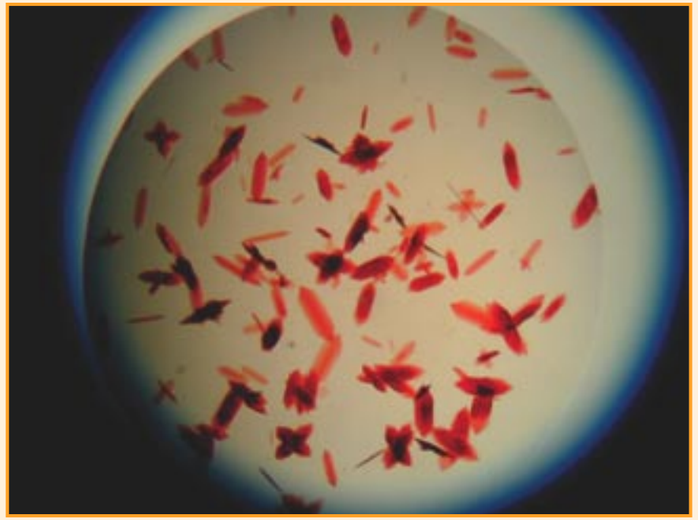

Figure 4.13. Crystallized cytochrome c3 from Desulfovibrio desulfuricans G20. (Image courtesy of J. Wall, Univ. Missouri.)

Enzymes that catalyze metal biotransformations are now being crystallized and analyzed by x-ray crystallography to determine the actual three-dimensional protein structure. The structure can provide information on how the enzyme works. Crystals have been obtained for the bacterial enzyme (MerB) that executes the first step in converting water-soluble methylmercury (MerB) to much less toxic metallic mercury, and for the tetraheme cytochrome c3 from *Desulfovibrio desulfuricans* that converts U(IV) to U(IV) (Figure 4.13).

SECTION V:

MICROBIAL PROCESSES

AFFECTING THE BIOREMEDIATION OF METALS AND RADIONUCLIDES

Sections II through IV described the basic ingredients for the bioremediation of metals and radionuclides — microbial metabolism, chemical speciation and valence status, and transport processes. Section V will describe how scientists and engineers believe these ingredients can be combined to bioremediate contaminated soils, sediments, and ground water. Bioremediation of metals and radionuclides relies on a complex interplay of biological, chemical, and physical processes. A fundamental, mechanistic understanding of the coupling between microbial metabolism, chemical reaction, and contaminant transport is beginning to develop, as well as how these activities could work together to bioremediate metals and radionuclides.

Microbes exist in complex biogeochemical matrices in subsurface sediments and soils. Their interactions with metals and radionuclides are influenced by a number of dynamic environmental factors, including solution chemistry, sorptive/reactive surfaces, and the presence or absence of organic ligands and reductants (Figure 5.1). Both biological and abiotic pathways contribute to the mineralization process.

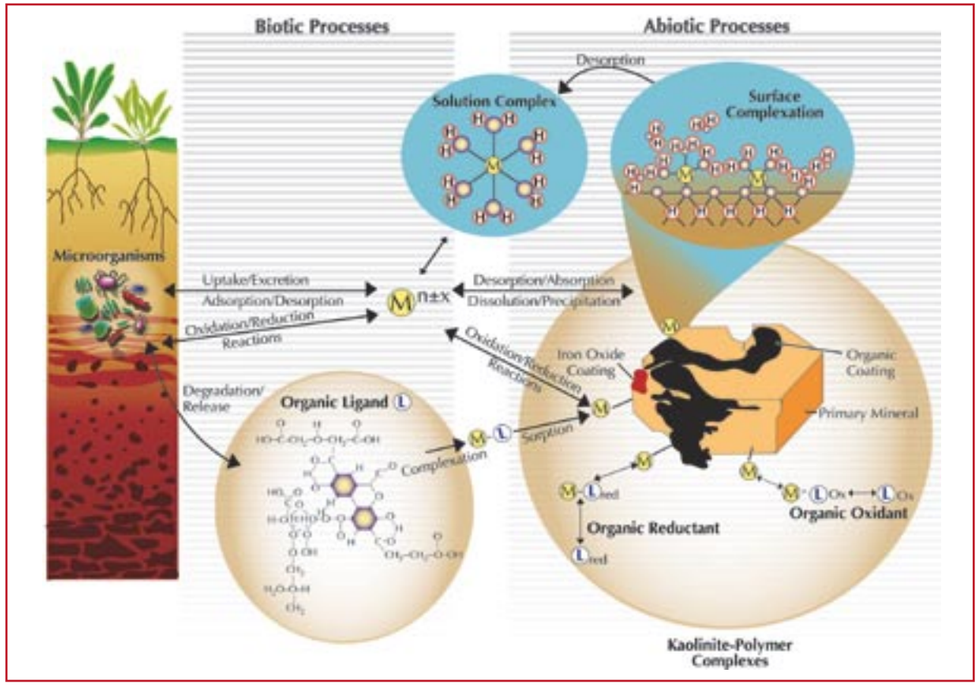

Figure 5.1. Abiotic and biotic mechanisms influence the fate of metals (M) in subsurface environments. From left to right: Organic material produced by microorganisms can act as ligands (L) and complex with metals, facilitating transport. These ligands can also be degraded by microbes to release the metal. Microbes can also directly adsorp/desorb, takeup/excrete, and oxidize/reduce metals. Oxidation and reduction will change the valence state of the metal either up or down ($M^{n\pm x}$). Abiotically, metals can form solution complexes or can complex with the surface of clays (kaolinite–polymer complexes). These mineral complexes can sorb metals either directly to the mineral or to organic or iron oxide coatings. Metals can also be directly oxidized or reduced to different valence states by the ambient redox conditions or by organic reductants (L_{red}) or oxidants (L_{ox}). Changing the valence state of metals will affect their sorption, mobility, precipitation, and toxicity. (Image courtesy of S. Fendorf, Stanford Univ.)

Iron cycling and associated changes in solid-phase chemistry have dramatic implications for the mobility and bioavailability of heavy metals and radionuclides. Coupled flow and water chemistry control the rate and solid phase products of iron hydroxide reduction and provide critical information in assessing the reactivity of reduced environments toward metal and radionuclide contaminants.

Bioremediation of soils, sediments, and water contaminated with metals and radionuclides can be achieved through biologically mediated changes in the oxidation state (speciation) of those contaminants — biotransformation. Changes in speciation can alter the solubility of metals and radionuclides, and therefore their transport properties and toxicity. The latter two characteristics can determine bioavailability, as discussed in Section I.

Resistance by subsurface microorganisms to the toxicity of heavy metals is critical for the bioremediation of contaminated subsurface sites. Remedial action depends on actively metabolizing microbes, and these microbes might be inhibited by high concentrations of toxic heavy metals. To assess metal resistance in microbes from the subsurface, two collections of bacteria (a total of 350 strains that were isolated from DOE's Savannah River and Hanford sites) were surveyed for their resistance to three metals that are of primary concern in contaminated subsurface sites. Of the 350 strains, 70% were resistant to lead, 45% were resistant to chromium, and 15% were resistant to mercury. How did resistance to metals evolve in the subsurface? One hypothesis is that resistance genes were laterally transferred among subsurface microbial populations. If confirmed, this process could be used to enhance metal resistance among microbes in bioremediated sites.

There are at least three types of microbial processes that can influence the toxicity and transport of metals and radionuclides: biotransformation, biosorption and bioaccumulation, and degradation or synthesis of organic ligands that affect the solubility of the contaminants. Each offers the potential for bioremediation of metallic and radioactive contaminants in the environment.

BIOTRANSFORMATION

Metal-reducing microorganisms can reduce a wide variety of multivalent metals that pose environmental problems at many DOE facilities. The heavy metals and radionuclides subject to enzymatic reduction by microbes include but are not limited to uranium (U), technetium (Tc), and chromium (Cr). Direct enzymatic reduction involves use of the oxidized forms of these contaminants as electron acceptors.

The oxidized forms of U, Tc, and Cr are highly soluble in aqueous media and are generally very mobile in aerobic ground water, while the reduced species are highly insoluble and often precipitate from solution. Direct enzymatic reduction of soluble U(VI), Tc(VII), and Cr(VI) to insoluble species has been documented and is illustrated in Figure 5.2.

Extracellular precipitation of metals and radionuclides has been demonstrated in a number of microbial isolates. For example, the precipitation of uranium on the cell surface of the bacterium *Shewanella* is shown in Figure 5.3. Metal-reducing organisms reduce uranyl carbonate, which is exceedingly soluble in carbonate-bearing ground water, to highly insoluble U(IV), which precipitates from solution as the uranium oxide mineral uraninite.

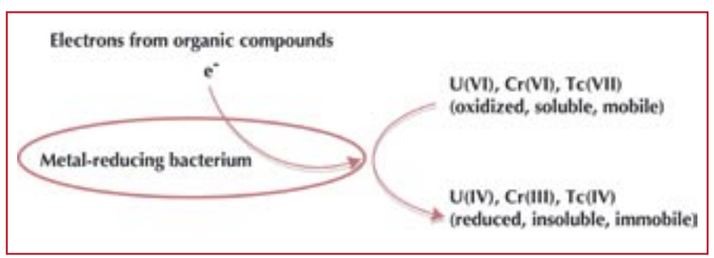

Figure 5.2. Direct enzymatic reduction of soluble heavy meals and radionuclides by metal-reducing bacteria. Nonhazardous organic compounds, such as lactate or acetate, provide electrons used by these microorganisms. Note, however, that if complexed, the reduced species may become mobile.

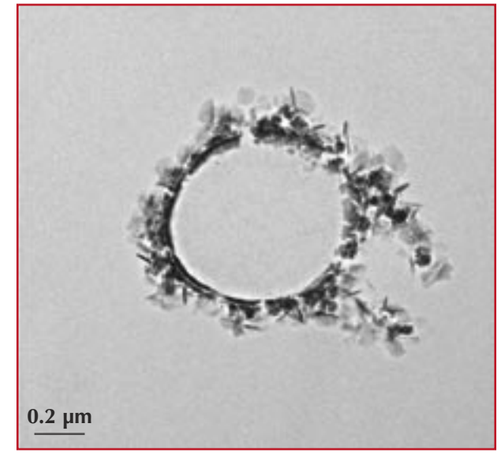

Figure 5.3. Microbes can play an important role in immobilizing radionuclides. This image shows a cross section of the bacterium *Shewanella* with uraninite precipitated on the cell surface. (Image courtesy of S. Fendorf, Stanford Univ.)

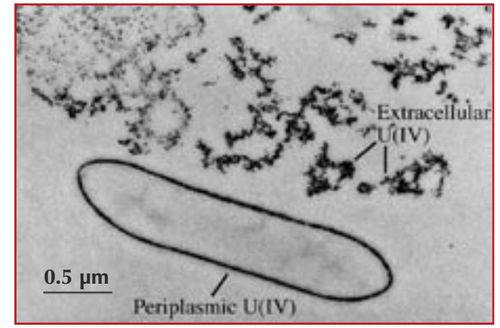

Figure 5.4. Transmission electron micrograph (TEM) showing extracellular and periplasmic U(IV) precipitates formed by enzymatic reduction of U(VI) by the subsurface bacterium *Geobacter sulfurreducens*. (TEM image obtained by S. Glasauer, Guelph Univ.)

Significant advances have been made in understanding the mechanisms of reduction of Fe(III), U(VI) and Tc(VII) in the subsurface bacterium *Geobacter sulfurreducens*, using the tools of biochemistry and molecular biology. A surface-bound *c*-type cytochrome of mass 40 kDa that is involved in the transfer of electrons to extracellular insoluble Fe(III) oxides has been identified and characterized. This protein is not required for the reduction of U(VI), suggesting that the mechanisms of Fe(III) and U(VI) reduction may be distinct. Another *c*-type cytochrome (9.6 kDa), found in the periplasm, appears to be required for U(VI) reduction (Figure 5.4). This protein is able to reduce U(VI) in vitro, and a mutant unable to synthesize the protein was unable to reduce U(VI) efficiently. Surprisingly, Tc(VII) is reduced by yet another mechanism, a periplasmic Ni/Fe-containing hydrogenase that uses hydrogen as the electron donor for metal reduction. Moreover, efficient indirect mechanisms may be important in immobilizing Tc in sediments wherein biologically reduced Fe(II) or U(IV) is able to transfer electrons directly to Tc(VII).

Technetium-99 is a major risk-driving contaminant at DOE's Hanford site, where ground water plumes are predicted to intersect the Columbia River in the future. Technetium-99 exists in soil and ground water primarily as the oxidized, mobile pertechnetate anion. Pertechnetate [$Tc(VII)O_4^-$] can be reduced to an immobile solid [$Tc(IV)O_{2(s)}$] directly by metal-reducing bacteria and by reaction with sorbed, biogenic Fe(II) resulting from the activity of metal-reducing bacteria. A remedial strategy could involve the reductive capture of $Tc(VII)O_4^-$ through strategic placement of a biostimulated zone of metal-reducing bacteria that generates Fe(II) in advance of the migrating contaminant plume. DOE ground water plumes with $Tc(VII)O_4^-$ may contain NO_3^- at concentrations greatly in excess of technetium-99. Because NO_3^- has the potential to react with biogenic Fe(II), it has been argued that NO_3^- would require expensive pretreatment to allow $Tc(VII)O_4^-$ immobilization. However, researchers have found that $Tc(VII)O_4^-$ can be selectively removed from high-NO_3^- water by reductive reaction with biogenic Fe(II). These results imply that the reductive immobilization of $Tc(VII)O_4^-$ as a remedial strategy is biogeochemically feasible.

A wide range of bacteria reduce the highly soluble chromate ion to Cr(III), which under appropriate conditions precipitates as $Cr(OH)_3$. A number of Cr(VI)-reducing microorganisms have been isolated from chromate-contaminated waters, oils, and sediments, including *Arthrobacter* sp., *Pseudomonas aeruginosa* S128, some anaerobic sulfate-reducing bacteria, and even several algae. Laboratory experiments with Hanford Site sediments showed that Cr(VI) concentrations in pore

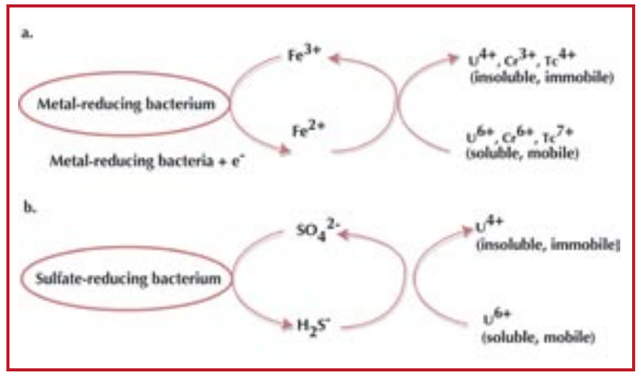

Figure 5.5. Indirect mobilization of heavy metals and radionuclides by (a) metal-reducing and (b) sulfate-reducing bacteria.

water decreased significantly (>66%) in a month-long incubation in the presence of nitrate and added dilute molasses as an electron donor. Thus, the addition of molasses to vadose zone sediments shows potential to decrease the transport of chromium and nitrate into underlying aquifers.

Although some microorganisms can enzymatically reduce heavy metals and radionuclides directly, *indirect reduction* of soluble contaminants may be possible in sedimentary and subsurface environments, although this has not been demonstrated under natural conditions, to date. This indirect immobilization could be accomplished by metal-reducing or sulfate-reducing bacteria. One approach would be to couple the oxidation of organic compounds or hydrogen to the reduction of iron [Fe(III)], manganese [Mn(IV)], or sulfur [S(VI) in the form of sulfate, SO_4^{2-}]. Iron(III) can be biologically reduced to Fe(II), Mn(IV) to Mn(II), and S(VI) (sulfate) to S(II) (hydrogen sulfide, H_2S). The reduced product might then, in turn, chemically reduce metals or radionuclides to yield separate or multicomponent insoluble species.

The most reactive of these reduced species are Fe(II) and H_2S. Ferrous iron [Fe(II)], which is generated by the enzymatic activity of iron-reducing and some fermentative bacteria, can reduce multivalent metals such as uranium, chromium, and technetium (Figure 5.5.a). The reduced forms of these metals are insoluble and can either precipitate as reduced oxide or hydroxide minerals, or coprecipitate with Fe(III) minerals that form during the reoxidation of Fe(II). In coprecipitation, elements are incorporated in metal oxide minerals as they precipitate from solution.

Sulfate-reducing bacteria also may be stimulated to produce a chemically reactive redox barrier (Figure 5.5.b). Hydrogen sulfide generated by sulfate-reducing bacteria could chemically reduce the contaminant to a form that would be stable for extended periods of time. A study of biofilms in a zinc and lead mine is a good example of indirect immobilization of heavy metals by sulfate-reducing bacteria. Sulfide produced by sulfate reducers in the film scavenged zinc and other toxic metals. X-ray fluorescence microbeam analysis revealed that zinc and small amounts of arsenic and selenium were extracted from ground water and concentrated in biofilms in zinc sulfide precipitates (Figure 5.6). Thus, microbial formation of sulfide deposits drastically decreased the migration of contaminant metals.

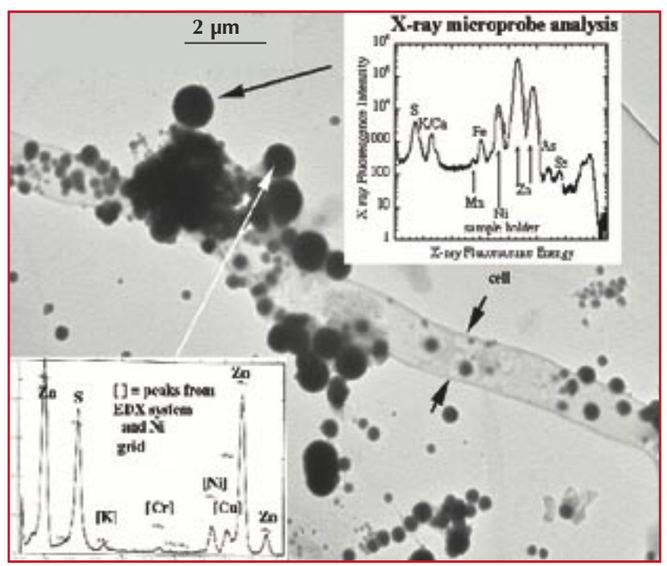

Figure 5.6. X-ray fluorescence trace metal microanalysis of sulfate-reducing bacteria. Results of electron (bottom left) and x-ray (top right) microprobe analysis of specific biomineralized zinc sulfide precipitates. The sensitivity of the x-ray microprobe enables identification of arsenic and selenium constituents in the zinc sulfide precipitate on the surface of a sulfate-reducing bacterium. (Image courtesy of K. M. Kemner, Argonne National Laboratory, and J. F. Banfield, University of California, Berkeley.)

Manganese(III/IV) oxides, which are common mineral phases in many soils and sediments, are also electron acceptors for metal-reducing bacteria. Manganese oxides are also relatively strong oxidants and can oxidize insoluble, reduced contaminants such as the mineral uraninite (UO_2), a common product of microbial uranium reduction. Differences in the solubility of oxidized Mn (insoluble) and U (soluble) challenge predictions of their biogeochemical behavior during in situ bioreduction. Results from laboratory experiments with the subsurface bacterium *Shewanella putrefaciens* CN32 showed that Mn oxides impeded the rate and extent of U(VI) reduction. In the absence of Mn oxides, CN32 quantitatively reduced U(VI) to U(IV), in the form of UO_2, a solid. The UO_2 was observed in regions external to the cell as well as in the periplasm, the region between the inner and outer membrane of the cell. In the presence of the Mn oxides, the reduced U resided exclusively in the periplasm of the bacterial cells (Figure 5.7). These results indicate that the presence of Mn(III/IV) oxides may impede the in situ biological reduction of U(VI) in subsoils and sediments. However, the accumulation of U(IV) in the periplasm indicates that the cell may physically protect reduced U from oxidation by Mn oxides, suggesting that extensive reduction of soil Mn oxides may not be required for reductive bioimmobilization of U(IV).

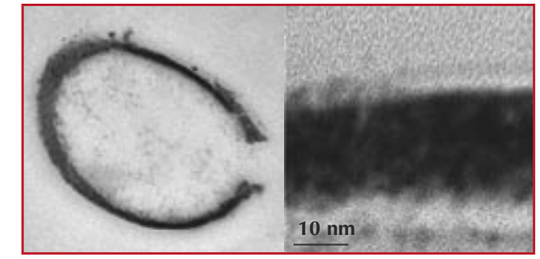

Figure 5.7. TEM images of unstained thin sections from *S. putrefaciens* CN32 cells incubated with H_2, as the electron donor, and U(VI) in bicarbonate buffer in the presence of Mn oxides such as bixbyite or birnessite exhibited an absence of fine-grained extracellular UO_2(s) and accumulation of UO_2(s) exclusively in the periplasm. (Images courtesy of J. Fredrickson of Pacific Northwest National Laboratory.)

Natural organic matter (NOM) may play a role in the reduction of contaminants such as Cr(VI) and U(VI) in subsurface environments. NOM consists of a mixture of organic compounds with different structures and functional groups. These groups include aromatic and phenolic moieties, carboxylic and heteroaliphatic hydroxyl functional groups, and free radicals. In the presence of a metal-reducing bacterium, NOM effectively mediated the transfer of electrons for the reduction of Fe(III), Cr(VI), and U(VI), although the reduction rate varied among different NOM samples and among contaminants.

BIOACCUMULATION AND BIOSORPTION

Microorganisms can physically remove heavy metals and radionuclides from solution through association of these contaminants with biomass. Bioaccumulation is the retention and concentration of a substance *within* an organism. In bioaccumulation, solutes are transported from the outside of the microbial cell through the cellular membrane, and into the cell cytoplasm, where the metal is sequestered.

Biosorption describes the association of soluble substances with the cell surface. Sorption does not require an active metabolism. The amount of metal biosorbed to the exterior of bacterial cells often exceeds the amount predicted using information about the charge density of the cell surface. Scientists have demonstrated that charged functional groups serve as nucleation sites for deposition of various metal-bearing precipitates.

Three possible nonreducing mechanisms of actinide–microbe interactions are shown in Figure 5.8. (The example shows sorbed U(VI), which appears to remain in its oxidized state.) These include: (1) sorption on cell surface sites; (2) additional surface complexation and precipitation of actinides; and (3) precipitation of actinides with

bacterial cell lysates. In Gram-positive bacteria, surface complexation occurs between organic phosphate groups in cell surface teichoic acid and U(VI). Uranium(VI)–phosphate solids are the least soluble of all the U(VI) solid phases. By contrast, Gram-negative bacteria appear to have a lesser ability to sorb U, possibly because they lack these cell-surface organic phosphate groups. One of the most common surface structures found in both Bacteria and Archaea is a crystalline proteinaceous surface layer called the S-layer. The S-layer appears to attenuate the sorption ability of Gram-positive bacteria.

Advanced analytical technologies — such as electron microscopy (EM), x-ray absorption near-edge structure (XANES), and extended x-ray absorption fine structure (EXAFS) spectroscopy — have enhanced our understanding of biosorption and bioaccumulation. EXAFS can be used to determine multiple species of actinides when relatively high concentrations are bound to the cell surface. X-ray diffraction (XRD) can also be coupled with EM to localize biosorbed/bioaccumulated substances on or within cells. Another technique, time-resolved laser fluorescence spectroscopy (TRLFS), can be used to identify the component of the cell surface responsible for actinide complexation when concentrations are low.

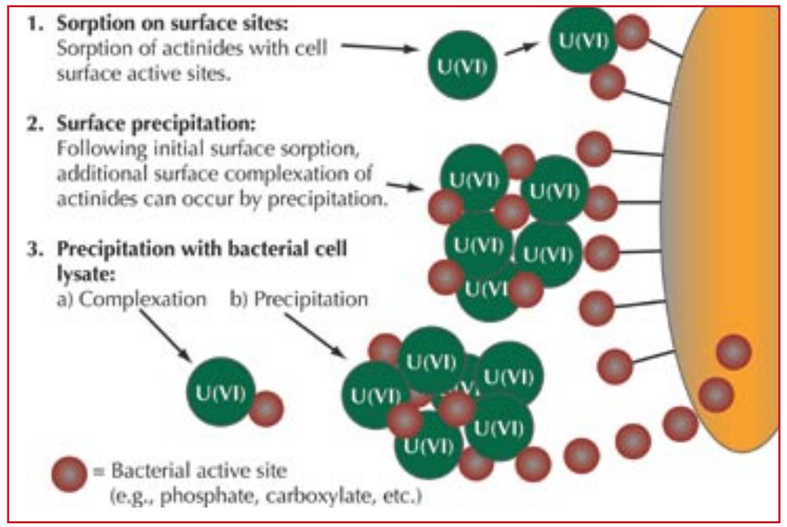

Figure 5.8. Three possible nonreductive mechanisms of bacterial cell surface interaction with U(VI). (Courtesy of H. Nitzche and T. Hazen, Lawrence Berkeley National Laboratory, and S. Clark, Wash. State Univ.)

SIDEROPHORE-MEDIATED UPTAKE BY MICROORGANISMS

In aerobic soils, iron exists primarily as Fe(III), which has low water solubility (~10^{-18}) and cannot be acquired as the free ion by soil microbes. To circumvent this problem, microbes produce siderophores, low-molecular-weight chelating agents that bind with iron and transport it into the cell through an energy-dependent process (Figure 5.9). Experiments have shown that various metals can form complexes with siderophores and that many of these complexes are recognized by cell uptake proteins. Study of siderophore complexation with actinides and the uptake of these complexes is an important component in the understanding of how microbes and actinides interact in the environment.

Researchers have now demonstrated that a microorganism can take up plutonium by the same mechanism it uses to take up iron. The common soil microorganism *Microbacterium flavescens* uses siderophores to obtain its nutritionally required iron. Bacteria were incubated with the siderophore desferrioxamine-(DF) bound with either plutonium [Pu(IV)], iron [Fe(III)], or uranium [U(VI), as UO_2^{+2}]. Using transport proteins, the cells took up the Pu–siderophore complex (Figure 5.9), although at a much slower rate than they took up the Fe–siderophore complex; however, they did not take up the U–siderophore complex. Only metabolically active bacteria were capable of taking up the Pu siderophore complexes, just as with Fe–siderophore uptake.

The two complexes [Pu(IV)–DF and Fe(III)–DF] mutually inhibit the uptake of one another, indicating that they compete for the same binding sites or transport mechanisms in the microbe. This is not surprising, because the structures of the Pu(IV)–DF and Fe(III)–DF complexes are similar, which suggests they could possibly be recognized by the same bacterial uptake system. These discoveries could have wide-ranging implications for future bioremediation efforts and for more accurate predictions of how plutonium and other actinides behave in the environment. Siderophore-mediated uptake and transport could be an important pathway for environmental mobility and Pu entry into the food chain.

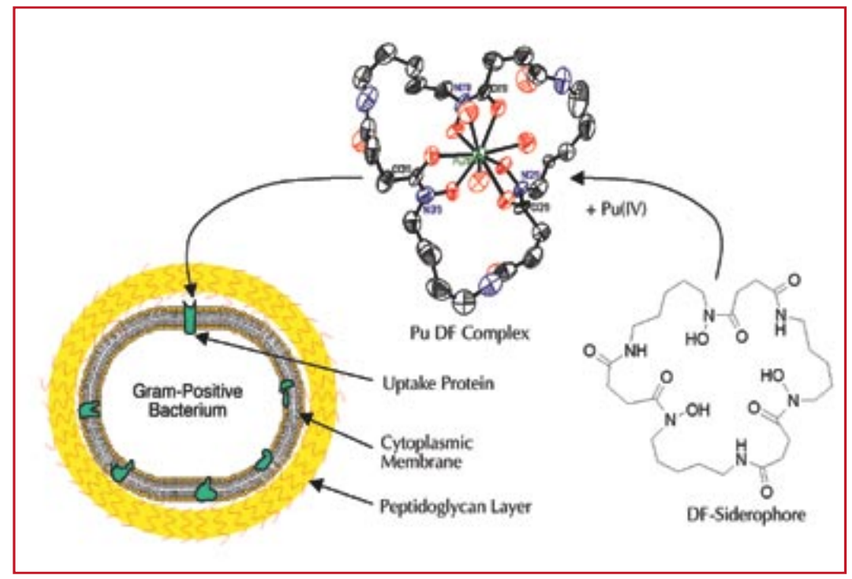

Figure 5.9. Siderophore-mediated Pu accumulation by *Microbacterium flavescens* (John et al., 2001).

MICROBES AND SYNTHETIC ORGANIC CHELATORS

Organic complexing agents can have a profound effect on the mobility of metals and radionuclides in subsurface environments. One type of complexing agent is called a chelator — an organic compound that forms two or more coordination bonds with a central metal ion. Heterocyclic rings are formed with the central metal atom as part of the ring. Synthetic chelators such as EDTA and NTA can form stable, soluble complexes with heavy metals and radionuclides. These chelators were commonly used as cleaning agents during industrial processing of nuclear fuels throughout the DOE complex

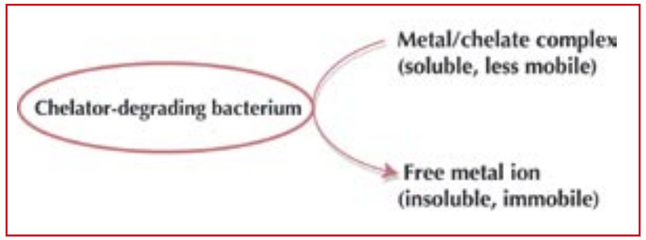

Figure 5.10. Immobilization of radionuclides and heavy metals by enzymatic degradation of organic chelators, such as EDTA and NTA.

and were sometimes co-disposed with metals and radionuclides. Metal–chelate complexes have entered the environment and may migrate in ground water. However, the migration of these complexes can be reduced by the biodegradation of the organic ligand (Figure 5.10). The resulting free metal ions are likely to adsorb to mineral surfaces or to form oxide mineral precipitates that would be less mobile in ground water. The degradation of organic chelators associated with metal or radionuclide contaminants, then, might achieve a desirable immobilization of contaminants in place.

Some of these chelators can be degraded by naturally occurring microorganisms. A number of EDTA- and NTA-degrading organisms have been isolated and identified. In one study, microbial degradation of EDTA by the environmental isolate BNC1 was influenced by the complexed metal. Cobalt(II)–EDTA, cobalt(III)–EDTA, and nickel(II)–EDTA complexes were not degraded, whereas copper(II)–EDTA and zinc(II)–EDTA complexes were. The genes and enzymes responsible for EDTA and NTA degradation have been identified, and the genes have been cloned and sequenced. All the genes necessary to code for degradation of EDTA and NTA occur together in a "gene cluster." Elucidating the genes and enzymes responsible for EDTA and NTA biodegradation will provide an understanding of the environmental and physiological controls on chelate degradation in bacteria, and provide gene probes for monitoring this process in the environment. Such fundamental research on the mechanisms of enzymatic degradation of synthetic chelators is expected to provide useful information for developing bioremediation strategies.

Metal-reducing bacteria also sometimes actually promote the mobilization of insoluble forms of some heavy metals and radionuclides. It has been demonstrated that metal-reducing bacteria can solubilize PuO_2, which is insoluble, in the presence of the synthetic chelator NTA. It is thought that the bacteria reduced the insoluble Pu(IV) to Pu(III), which was then complexed by NTA. This process may provide a means of mobilizing Pu from contaminated soils and sediments, and could be a step in the removal of this highly toxic radionuclide from the environment. However, this approach has not been tested in the field.

Under anaerobic conditions, uranyl–citrate complexes are not metabolized. However, in the presence of an electron donor, U(VI) was reduced to U(IV) and remained in solution as the U(IV)–citrate complex. These results show that complexed uranium is readily accessible to anaerobic microbes as an electron acceptor, despite their inability to metabolize the organic ligand complexed to the actinide.

Organic acids formed by the metabolic activity of microorganisms can lower the pH of the system to values that interfere with the electrostatic forces that hold heavy metals and radionuclides on the surface of iron or manganese oxide minerals. Displacement of cations by hydrogen ions may lead to the solubilization of the surface-associated metal or radionuclide. In some cases, the organic metabolites also serve as complexing agents that can form soluble metal–ligand complexes. These complexing agents (which include dicarboxylic acids, phenolic compounds, ketogluconic acids, and salicylic acids) have been shown to promote the dissolution of a wide range of heavy metals and radionuclides, including PuO_2. Therefore, biogenic production of complexing agents can accelerate the movement of metals in soils and sediments.

SECTION VI:

FIELD RESEARCH
ON BIOREMEDIATION OF METALS AND RADIONUCLIDES

Important information on the fundamental principles of bioremediation can be gained by careful laboratory studies with individual microorganisms in culture and with environmental samples in laboratory microcosms that simulate natural conditions. However, in some cases, this information does not readily translate to the field, due to the complexity of natural environments. For this reason, hypothesis-driven research performed *in the field* is of critical importance to the science of bioremediation. Field research benefits from supportive laboratory-based studies. Some of the field research questions currently being posed include: What are the structure, function, distribution, and activity of microbial communities in the subsurface? How can in situ biotransformation potential be assessed? What nutrients might stimulate biotransformation? Answering these questions requires the involvement of multiple scientific disciplines, including microbiology, ecology, geochemistry, hydrology, environmental engineering, and numerical modeling, just to name a few.

FIELD RESEARCH SITES

Field research requires sites that are well characterized in terms of their hydrology and geology, and are accessible for collection of samples of sediments and ground water. Two such sites are the NABIR Field Research Center in Oak Ridge, Tennessee, and the Uranium Mill Tailing Remedial Action (UMTRA) sites.

The NABIR Field Research Center (FRC) provides a site for investigators to conduct research and obtain samples related to in situ bioremediation of metals and radionuclides. The FRC is located within the Y-12 National Security Complex area on DOE's Oak Ridge Reservation (Figure 6.1). The geology of the site, which

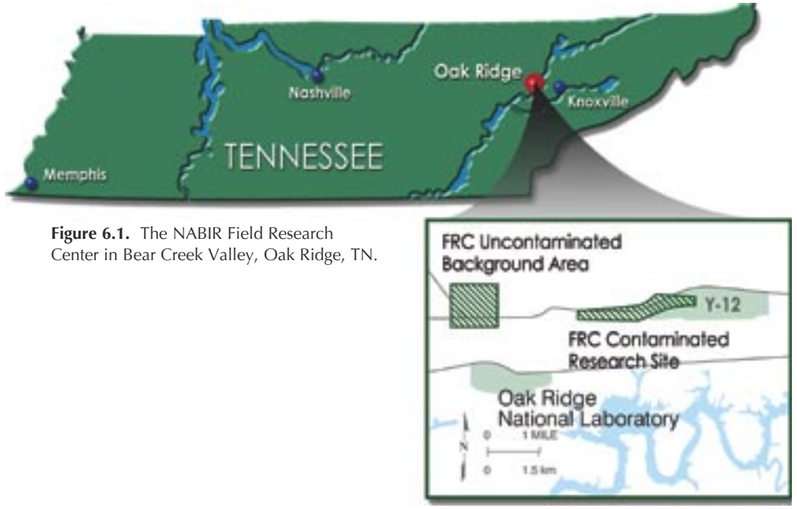

Figure 6.1. The NABIR Field Research Center in Bear Creek Valley, Oak Ridge, TN.

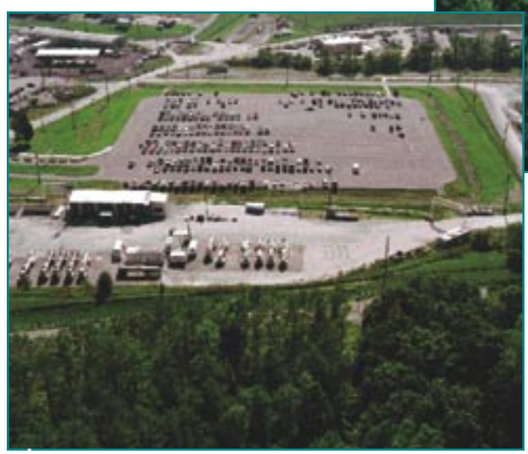

Figure 6.2. (a) Source of contaminants: the S-3 Disposal Ponds (in operation from 1951 to 1983, and neutralized in 1984). (b) The area was capped in 1988 and paved as a parking lot.

lies in the Bear Creek Valley, is characterized by unconsolidated residuum overlying Nolichucky shale. The depth to ground water is typically <5 m. The FRC includes a 163 hectare contaminated area and a background area that provides for comparative studies in an uncontaminated area with a similar hydrogeological setting.

The source of contamination is commingled ground water plumes that originated from the S-3 disposal ponds (Figure 6.2). These ponds operated from 1951 to 1983 and received over 2.5 million gallons of mixed waste each year. The wastes contained nitrate, uranium, technetium-99, metals, and volatile organic compounds. The pH of the ponds was less than 2.0. The ponds were neutralized in 1984 and capped in 1988. The initial focus of research at this site is on in situ biostimulation to promote immobilization of uranium in the presence of high levels of nitrate in unconsolidated residuum and fractured rock. Conceptual models have been developed of the transport of nitrate and uranium at this site (Figure 6.3).

The Uranium Mill Tailings Remedial Action (UMTRA) sites are former uranium mill processing sites located in several states (Figure 6.4). At these sites, uranium was once milled to use in the federal government's national defense programs or at nuclear power plants. When processing mills shut down, large piles of the sand-like tailings remained, containing approximately 85 percent of the radioactivity of the ore. Remedial action consists of minimization or elimination of potential surface and ground water health hazards resulting from exposure to residual radioactive materials.

Consolidating the tailings and isolating them from the environment in engineered disposal cells was the first step in this remedial effort. Once DOE eliminated the source of contamination, attention centered on the ground water pollution beneath the sites and the best approach to ensure compliance with U.S. Environmental Protection Agency (EPA) ground water standards. The types and concentrations of contaminants vary among the sites, but uranium is a common contaminant. One or more of the following contaminants have been measured in ground water samples at each site: arsenic, barium, cadmium, chromium, lead, molybdenum, nitrate, radium-226 and -228, selenium, uranium, and vanadium. NABIR researchers have

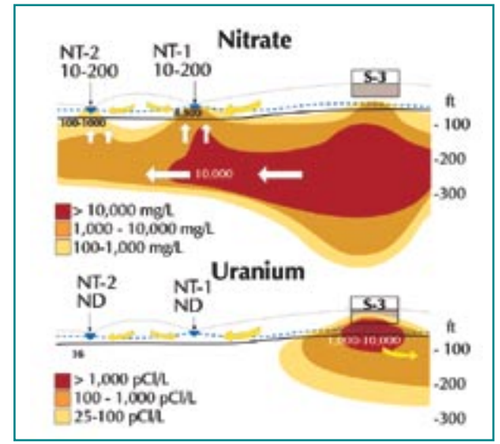

Figure 6.3. Cross section showing transport of nitrate and uranium from the S-3 ponds in the subsurface. NT-1 and NT-2 stand for North Tributary 1 and 2, respectively. They are tributaries feeding Bear Creek. (Images for Figures 6.1–6.3 courtesy of D. Watson, Oak Ridge National Laboratory.)

collaborated with the UMTRA Ground Water Project to identify the dominant electron-accepting processes for in situ biotransformation of metals and radionuclides in ground water and sediments at several of these sites. Using a combination of phospholipid fatty acid (PLFA) biomarkers and nucleic acid-based (denaturing gradient gel electrophoresis) analysis of the microbial communities, it was shown that the subsurface microorganisms at several UMTRA sites were diverse and had a broad range of metabolic capabilities.

Microcosm studies using sediments from UMTRA sites showed that stimulating the activity of dissimilatory metal-reducing microorganisms in uranium-contaminated subsurface sediments could effectively precipitate uranium out of the ground water. Uranium precipitation takes place concurrently with microbial Fe(III) reduction and results from reduction of soluble U(VI) to insoluble U(IV). The reduction of U(VI) is associated with a microbial community shift in which members of the *Geobacteraceae* family dominate in the sediments. Analysis of the composition of the microbial community demonstrated that, whereas *Geobacteraceae* comprised less than 5% of the microbial community prior to the stimulation of metal reduction, they accounted for over 40% of the microbial community during the metal-reduction phase. These studies have led to the design of a field experiment for in situ uranium bioremediation at the Old Rifle, Colorado, UMTRA site, discussed below.

Figure 6.4. Map showing location of UMTRA sites in the U.S. (Courtesy of Pacific Northwest National Laboratory.)

Mathematical Modeling of Subsurface Processes

Mathematical models are powerful tools for designing bioremediation strategies, because they can deal with the multiple processes found at a hazardous waste site. Models vary in complexity, but should always include the most important geochemical, microbiological, and hydrological processes. The model's accuracy depends not only on including the right processes and an accurate mathematical description for each of these processes, but on many inputs that have to be either measured or estimated for the proper field conditions. These inputs include a physical and a geochemical description of the ground water environment, as well as accurate rates of reactions. Microbiological processes need to be examined as well, in particular, the structure and function of the subsurface microbial communities.

For a biostimulation experiment, the injection process needs to be determined: How much acetate needs to be injected into the ground water? Where should the injection wells be placed? Where will the uranium react and to what degree? How long can this process go on before some important nutrients are exhausted? How stable will the precipitated uranium be after the remediation process is discontinued?

Modeling, field measurements, and laboratory experiments often progress in a cyclic fashion, as progress and new findings in one area drive improvements in the next. For example, model predictions can refine field sampling and new biogeochemical findings can drive the improvement of models.

Examples of Field Research on Bioremediation of Metals and Radionuclides

Push-Pull Studies

Single-well, "push-pull" tests can be used to determine kinetics of microbially mediated uranium reduction in situ. The push-pull test methodology consists of the pulse-type injection ("push") of a prepared aqueous test solution into the saturated zone, a discrete time period for interactions, followed by the extraction ("pull") of the test solution/ground water mixture from the same location (Figure 6.5). The injected test solution usually contains various combinations of tracers (such as Br^-) and electron donors and/or acceptors, depending on the objective of the individual test. By monitoring the changing composition of the injected test solution through time, the kinetics of electron acceptor and electron donor utilization may be quantified.

Push-pull studies allow researchers to probe the native subsurface microbial communities in situ to assess their ability to reduce uranium in subsurface environments. For example, a range of organic compounds that serve as electron donors can be provided to determine which one might be most effective in stimulating the growth and activity of metal-reducing microorganisms. Push-pull studies have been successfully conducted at a number of contaminated sites, including UMTRA sites and the NABIR Field Research Center.

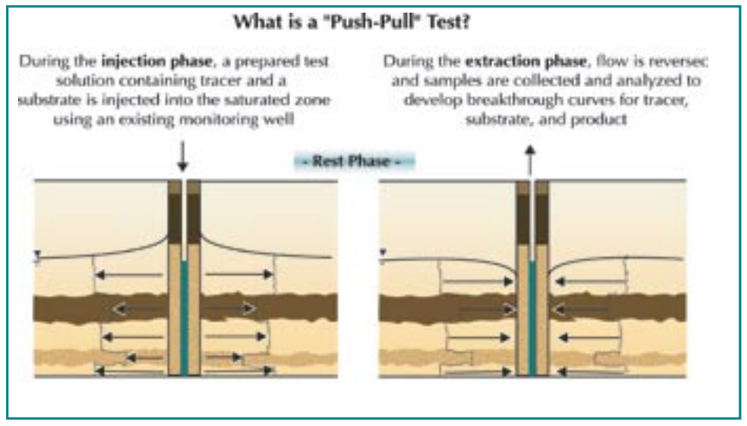

Figure 6.5. Diagram showing concept of "push-pull" experiments in subsurface environments. (Courtesy of J. Istok, Oregon State Univ.)

Push-pull studies at an UMTRA site showed the potential for stimulating in situ removal of soluble U(VI) upon the injection of acetate into the saturated zone. Uranium(VI) concentrations decreased approximately 30–60% after injection of the electron donor, acetate. The observed loss of U(VI) usually coincided with the production of Fe(II). These results demonstrate the potential to stimulate removal of soluble U(VI) from ground water under iron-reducing conditions in the subsurface.

Biostimulation

The addition of nutrients to stimulate the in situ immobilization of metals and/or radionuclides is a form of biostimulation. Biostimulation can lead to creation of a permeable treatment zone in the aquifer that removes the metals and radionuclides from the aqueous phase before they may impinge on sensitive water supplies. If the ground water is below approximately 15 meters, the treatment zone must take advantage of in situ processes, because it becomes impractical to excavate and place barrier materials below these depths.

The first step of a field biostimulation experiment begins in the laboratory and consists of microcosm studies to confirm the potential for stimulating biological reduction and immobilization of the contaminants through the addition of organic substrates. The relative efficiency of a range of organic substrates (e.g., lactate, acetate, glucose) for biostimulation might also be tested in the microcosms. For field studies, detailed hydrologic models are coupled with geophysical, geochemical, and biological process level information to design treatment systems. (Figure 6.6 shows a strategy for a field biostimulation experiment.) Carbon sources and electron donors must be delivered to the specific

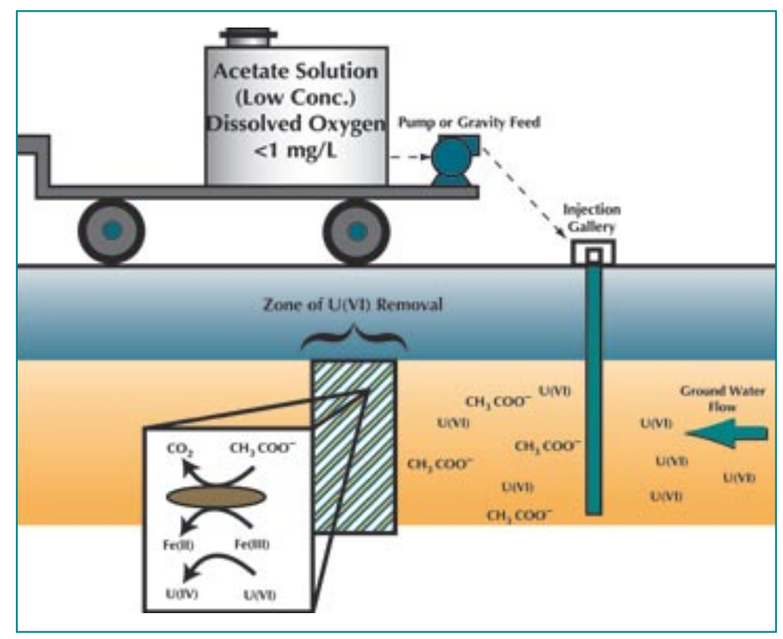

Figure 6.6. Strategy for bioremediation of a uranium-contaminated aquifer. (Graphic courtesy of D. Lovley and T. Anderson, Univ. Massachusetts.)

location in the subsurface that is being treated. In metabolizing the organic carbon, indigenous heterotrophic microorganisms consume available oxygen and facilitate the growth of anaerobic metal reducers. Biological reduction of metals and radionuclides (either directly or indirectly) results in removal of the soluble contaminant from the aqueous phase. A careful monitoring plan should be implemented to determine the long-term effectiveness of the treatment and the potential for reoxidation (and thus remobilization) of the contaminants.

A successful biostimulation experiment for immobilization was performed at an UMTRA site in Rifle, Colorado. Laboratory studies using native sediments clearly showed that the addition of acetate as an electron donor resulted in precipitation of U(IV). Engineers, hydrologists, geochemists, and microbiologists worked together to design a biostimulation experiment in which over 1,000 gallons of acetate were added to a uranium-contaminated site. Over a period of about two months, concentrations of U(VI) in the ground water decreased as acetate was consumed. The next step is to address competition by other microorganisms for electron donors and the long-term stabilization of the precipitated uranium at the UMTRA site.

Combining Ex Situ and In Situ Methods

Contaminants in the environment rarely occur alone; mixtures of wastes are far more common. Environmental conditions can be so complex that several methods of waste treatment might need to be coupled to remove or immobilize the contaminants effectively. One such example is a contaminant plume in a source zone area adjacent to the S-3 Waste Ponds at the NABIR FRC in Oak Ridge. The pH of the ground water in this zone averaged about 3.5. Contaminants at this part of the FRC included high levels of uranium, nitrate, aluminum, and chlorinated solvents. Acidic conditions correlated with low microbial diversity, and the high nitrate levels inhibited U(VI) reduction because nitrate is a thermodynamically preferable electron acceptor. Through denitrification, nitrate in subsurface environments can be biologically converted

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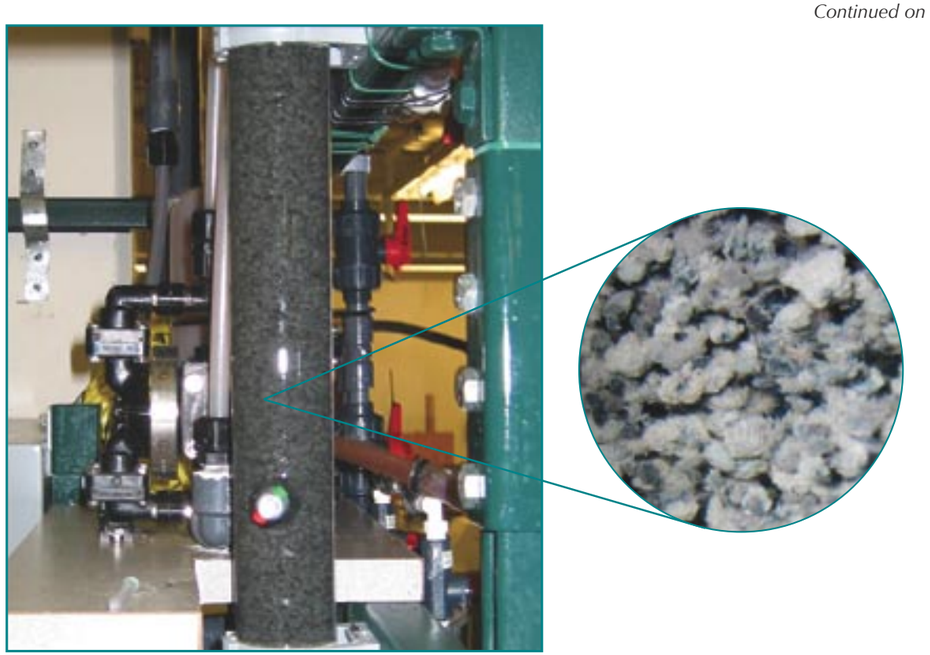

Figure 6.7. Left, pilot-scale fluidized bed reactor (FBR) is a glass column containing fluidized activated carbon particles. FBRs can be used for above-ground removal of nitrate and other contaminants. Right, a close-up of biofilms covering the FBR particles; biofilm microbes remove nitrate via denitrification. (Photos courtesy of M. Gentile and C. Criddle, Stanford Univ.)

The Importance of Scale

Natural geologic media have a hierarchical structure that controls the physical processes of transport and reactions. Figure 6.8 illustrates four scales that might influence bioremediation in porous media. Phenomena at the very smallest scales of interest can have a dramatic impact at the large scales that typically dominate environmental remediation problems. For example, processes at the cellular level (scale I, Figure 6.8) occur at characteristic length scales that might be on the order of micrometers (10^{-6} m). The effects of these processes are often observed in the field, as evidenced by microbial changes that occur in a contaminant plume (e.g., the biotransformation of metals; redox reactions that lower the Eh) as it moves through the subsurface.

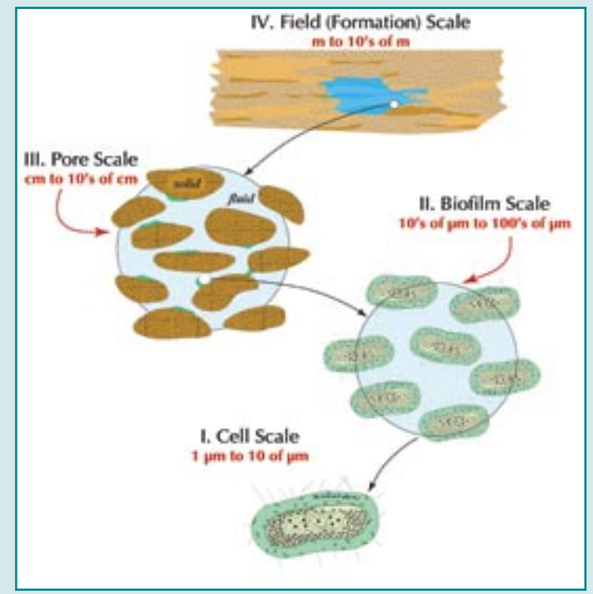

Figure 6.8. Hierarchical representation of the length scales relevant to bioremediation in the field. (Image courtesy of B. Wood, Oregon State University.)

As the scale increases from microbial cell to the field, processes that are manifest at increasingly large length-scales can influence the fate of contaminants in the subsurface. At scale II, microbial cells have proliferated to form a biofilm. Mass transfer and reactions within a biofilm are distinctly different from those of a single cell. For example, within a biofilm, the rates of transport of a contaminant may be affected by extracellular polymeric substances produced by microorganisms. At scale III, a complex pore network comprises the subsurface matrix. This porous network can be chemically heterogeneous (providing spatially variable geochemical and microbiological conditions within the pore space). The network increases the spreading of contaminants because of the tortuous pathways that the fluid follows as it flows through the network. At the largest scale of interest (scale IV), heterogeneities in the geological materials can influence both the types of microbial communities that form there (by altering local geochemical conditions) and the movement of contaminants in the ground water as they flow through the sequence of more and less permeable materials.

Upscaling is a process by which researchers take into account small-scale processes of interest when making decisions and observations at substantially larger scales such as encountered in field remediation problems. For example, it might be possible to show via upscaling that certain bulk parameters that are easily measured can represent the essential features of the multiscale processes that occur in the field. This would allow scientists and researchers to make predictions that are influenced by knowledge of detailed processes within cells themselves, but would require only information that could be practically measured.

Many reactions in soils and sediments are diffusion-limited, i.e., are controlled by the speed with which substrates move through pore water toward other dissolved reactants, toward water–mineral interfaces, or toward microbial surfaces. However, transport and reactions occurring within the diffusion-controlled domains that often make up most of the subsurface are commonly only inferred or assumed. Direct measurements within sediments are needed to understand biogeochemical processes, although such measurements may be difficult to make.

ENVIRONMENTAL MONITORING:
KEEPING AN EYE ON THE SUBSURFACE

Environmental cleanup requires up-to-date knowledge of the amount and behavior of water underlying a contaminated site, its physicochemical status (pH and Eh), and the types and concentrations of contaminants dissolved within. Ideally, this knowledge would be available instantaneously. This environmental information can provide useful clues about both contaminant speciation and the identity of microorganisms present. Remediation plans include a monitoring component that allows quantitative assessment of the physical and chemical condition of subsurface environments.

Ground Water Monitoring. This method is often carried out above ground by analysis of water pumped to the surface from extraction wells. These wells are distributed throughout the site of concern, in what is known as an onsite wellfield. Wellfield design is a critical portion of monitoring plans, as is sampling strategy (measurement location and frequency). Offsite wells, in unimpacted areas, are also needed to establish "background" levels for comparison. Sampling locations are carefully identified, because creation of each extraction well involves drilling a borehole — sometimes hundreds of feet deep — to reach the aquifer. Understanding of the hydrogeologic setting can be obtained prior to drilling via separate geophysical measurements. Key parameters to monitor include ground water levels and flow patterns, contaminant plume and related geochemical characteristics, and microbial presence, activity, and community composition.

Subsurface Geochemistry. To determine whether the extent, concentration, or movement of contaminant plumes is modified following an in situ experiment, scientists first need to know the baseline geochemical characteristics of the subsurface environment. Typical baseline geochemical analyses include direct measurement of parameters such as pH, Eh, dissolved oxygen, and temperature in the field using portable equipment, as well as extraction of ground water and/or sediment samples from the field site and subsequent analyses using laboratory-based equipment or techniques (e.g., inductively coupled plasma-mass spectrometry, kinetic phosphorescence analysis, time-resolved laser-induced fluorescence, ion chromatography or liquid scintillation analysis). Laboratory-based analyses include determinations of the concentrations of the contaminant(s) of interest (e.g., nitrate, U(VI), Cr(VI)), the extent to which other electron donors and acceptors are available (e.g., dissolved organic and inorganic carbon, sulfate, sulfide, iron speciation, manganese, ammonium), and perhaps other ground water or sediment-related parameters.

Once the baseline geochemical characteristics are defined, scientists can determine the extent to which manipulation of the subsurface causes subsequent changes in contaminant mobility and microbial community structure. Influences on contaminant transport can often be deduced by combining ground water monitoring data — specifically, pH, and Eh — with knowledge of the hydrogeologic setting (e.g., lithology) and physicochemical information about the contaminant(s) involved. By monitoring ground water data, researchers can determine whether the contamination comes from a single point source or from multiple loci.

Microbial Distribution, Activity, and Community Composition. The distribution, activity, and composition of subsurface microorganisms and the extent of interaction with contaminants of concern may be measured indirectly through study of substrate consumption or metabolite production. In the case of organic compounds, substrate disappearance is measured through traditional ultraviolet/visible spectroscopic, gas/liquid chromatographic, or radiotracer techniques. Appearance of metabolic intermediates or end products (including CO_2) is measured in the same manner. Microorganisms themselves can be observed via sophisticated imaging techniques such as confocal, fluorescence, or electron microscopy. Imaging techniques can be linked with x-ray methodologies to derive additional information about metal–microbe interactions.

New Assays for Monitoring Microbial Communities and Contaminants. *"Bio Trap Biosensors"* are a new approach for rapid monitoring of microbial communities in the subsurface. A sterile nonmetallic surface is provided for indigenous bacteria to colonize when suspended through a borehole. Microbes colonizing the traps from the ground water are recovered and analyzed for their biomarkers to assess the viable community biomass, composition, and nutritional status, using tandem mass spectrometry. These biomarkers also include respiratory quinones that provide information on the availability of oxygen to the subsurface microbial communities.

DNA Based Biosensors consist of small pieces of DNA molecules selected from a large pool of DNA molecules (>hundreds of trillions!) for their ability to bind specific metals. To increase the sensitivity of the sensor, a fluorescent tag can be attached. These sensors can be used to detect the concentrations of metals in subsurface environments.

Immunosensors have been developed for detecting U(VI), heavy metals, and chelators. The assay is based on using fluorescently labeled antibodies as biological recognition units. This approach provides a high specificity and sensitivity of detection, and a field-portable prototype instrument to measure concentrations of uranium in ground water is being tested and validated.

DNA Microarray Technologies are being developed for detecting specific types of microorganisms in the environment. Microarrays are tools based on the tendency of nucleic acids to bind or hybridize with complementary sequences. For example, a short segment of DNA (i.e., an oligonucleotide) can be used to detect specific 16S ribosomal RNA (rRNA) genes, which are present in all bacteria and indicate the type of bacterium. (Figure 6.9).

Environmental monitoring also plays an important role in long-term stewardship of the sites. Whether the bioremediation strategy is natural attenuation or biostimulation to immobilize contaminants, it is critical to ensure that the contaminants remain immobilized over time and do not pose a risk to humans or the environment.

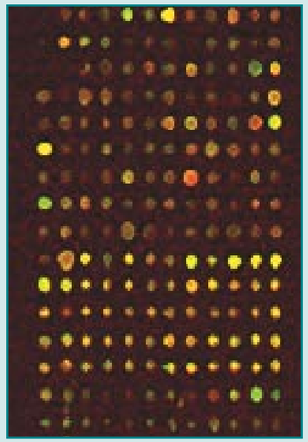

Figure 6.9. A DNA-based microarray for analysis of microbial communities. Thousands of DNA probes can be microscopically arrayed on an adherent surface. DNA from environmental samples is hybridized to the attached probes. In this case, whole genomic DNA from reference organisms serve as probes. The colors of the dots reflect degree of hybridization and therefore similarity of unknown microorganisms to probes. (Image courtesy of J. Zhou, Oak Ridge National Laboratory.)

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through a series of intermediates to N_2 gas. However, the concentrations of nitrate at some parts of the FRC are extremely high (up to 50 g/L). An in situ denitrification process was predicted to result in large amounts of N_2 gas and biomass, leading to possible aquifer plugging.

Therefore, researchers designed a bioremediation strategy that involved both ex situ and in situ treatments. The ex situ treatment allowed for neutralization of the pH (and subsequent removal of precipitated solids) and the removal of nitrate by denitrification. The ex situ treatment is a denitrifying fluidized bed reactor (FBR) in which microorganisms in biofilms attached to particles remove nitrate. A pilot-scale denitrifying FBR is shown in Figure 6.7.

The effluent from the ex situ FBR may contain residual uranium. By recirculating the effluent into the aquifer, the residual uranium can be removed in a downstream treatment zone through bioreduction by indigenous microorganisms. Thus, a two-stage system was designed with an ex situ treatment for removal of nitrate, acidity, aluminum, chlorinated solvents, and some uranium, followed by downstream in situ treatment for removal of the remaining, low levels of uranium.

Rewards of Field Research

Fundamental knowledge is needed to understand the biogeochemical processes that determine the fate and transport of metals and radionuclides in complex subsurface environments. Field research is critical to this understanding because it bridges the gap between small-scale laboratory studies and full-scale field implementation of new technologies. Too often, new technologies may be destined for failure because of the lack of understanding of the basic biogeochemical processes that control contaminants in situ. The ability to immobilize contaminants in the subsurface, combined with long-term stewardship, offers a new and cost-effective tool for cases where existing technology is not sufficient.

The Future of Natural and Accelerated Bioremediation Research

Today's scientific discoveries will lead to tomorrow's solutions to environmental problems. Basic research on bioremediation of radionuclides and metals is of paramount importance to the development of new strategies and technologies to solve critical environmental problems. While bioremediation research will have an enormous impact at contaminated DOE sites, the knowledge gained will also be widely applicable to industrial and military sites with metal contamination. Moreover, a wide range of "spin-offs" from this basic research is anticipated. For example, researchers have recently demonstrated the feasibility of using *Shewanella* isolated from a subsurface environment for rapid synthesis of pharmaceuticals used in nuclear imaging. Thus, basic research may impact more than one field, in this case, both environmental remediation and medical science.

NABIR is a dynamic program with ongoing new discoveries and scientific breakthroughs. This primer has attempted to capture just a few of the highlights of a complex, multifaceted program that continues to grow and evolve with time. It is anticipated that the exciting results from this important area of research will serve as the basis for future strategies and technologies in the field of bioremediation. For a complete list of published papers and the latest NABIR Program information, go to: http://www.lbl.gov/NABIR.

GLOSSARY

Abiotic: Occurring without the involvement of microorganisms.

Absorption: The process of taking up, absorbing, or being absorbed.

Accelerated bioremediation: Bioremediation accelerated beyond the normal actions of the naturally occurring microbial community and chemical and geological conditions, usually by the addition of nutrients or specialized microbes.

Actinide: A radioactive element in the series of elements beginning with actinium (89) and ending with lawrencium (103).

Adsorption: The adhesion of molecules (in a thin layer) to the surfaces of solid bodies or liquids with which they are in contact.

Advection: The process by which solutes are transported by the bulk motion of the flowing ground water.

Aerobic: Living, active, or occurring in the presence of free oxygen.

Algae: Photosynthetic eukaryotic unicellular and simple multicellular microorganisms.

Anabolism: The sequences of enzyme-catalyzed reactions by which essential molecules are formed in living cells; also known as biosynthesis.

Anaerobic: Living, active, or occurring in the absence of free oxygen.

Anion: A negatively charged ion.

Annotation: Assignment of function to genes identified by genomic sequencing.

Anthropogenic: Derived from human sources.

Aquifer: Stratum of permeable rock, sand, or gravel that can store and supply ground water to wells and springs.

Archaea: A group of prokaryotic single-celled microorganisms that constitute the recently recognized Archaea phylogenetic domain. Archaea can be distinguished from Bacteria in that their cell walls do not have murein, a peptidoglycan-containing muramic acid. Another unique feature of Archaea is the presence of isopranyl ether lipids in their cell membranes. The Archaea domain includes the methanogens, most extreme halophiles (needing salt for growth), certain sulfate reducers, hyperthermophiles (optimum growth temperature of 80°C or higher), and the genus *Thermoplasma*.

Assimilative metabolism: The reduction of inorganic compounds for use as a nutrient source.

ATP: Adenosine triphosphate, the principal energy carrier of the cell.

Autotroph: An organism able to utilize carbon dioxide as a sole source of carbon.

Bacteria: A group of prokaryotic single-celled microorganisms that constitute the Bacteria phylogenetic domain. Unlike Archaea, their cell walls have murein, a peptidoglycan-containing muramic acid. Bacteria may have spherical (coccus), rod-like (bacillus), or curved (vibrio, spirillum, or spirochete) bodies. They inhabit virtually all environments, including soil, water, organic matter, and may be associated with eukaryotes.

Bioaccumulation: Intracellular accumulation of environmental pollutants, such as heavy metals, by living organisms.

Bioaugmentation: The addition of microorganisms to the environment.

Bioavailability: The accessibility of chemical compounds in the environment to an organism or organisms.

Biobarrier: A biologically active zone that is placed in the subsurface perpendicular to the normal flow of a contaminant plume so that the contaminant can be adsorbed and biologically degraded.

Biodegradation: The breakdown of organic materials into simpler components by microorganisms or their enzymes.

Bioinformatics: The management, manipulation, and use of data derived from sequencing of genes and whole genomes.

Biomass: The amount of living matter present in a particular habitat.

Bioreactor: Vessel or tank in which whole cells or cell-free enzymes transform raw materials into biochemical products and/or less undesirable byproducts.

Bioremediation: The use of organisms (often microorganisms) to biodegrade or biotransform hazardous organic contaminants or biotransform hazardous inorganic contaminants to environmentally safe levels in soils, subsurface materials, water, sludges, and residues.

Biosorption: Sorption of a molecule by a microorganism.

Biostimulation: Addition of nutrients, oxygen, or other electron donors and acceptors so as to increase microbial activity and biodegradation.

Biotransformation: Alteration of the structure of a compound by a living organism or enzyme.

Bond: An attractive force that holds together the atoms, ions, or groups of atoms in a molecule or crystal.

Carcinogen: A substance or agent that initiates malignant tumor formation.

Catabolism: The biochemical processes involved in the breakdown of organic or inorganic compounds, usually leading to the production of energy. Important for bioremediation because contaminants may be transformed or degraded by microorganisms during catabolism.

Catalyst: A substance that activates a chemical reaction and is not itself changed in the process.

Cation: Positively charged ion.

Cell membrane: The permeable membrane surrounding the cell's cytoplasm; also called cytoplasmic membrane.

Cell wall: The layer or structure that lies outside the cell membrane, supporting and protecting the membrane and giving the cell shape.

Chelate: Any of a class of relatively stable coordination compounds consisting of a central metal atom attached to a large molecule, called a ligand, in a cyclic or ring structure.

Chelator: An agent that causes formation of a chelate.

Chemolithotroph: An organism that obtains its energy from the oxidation of inorganic compounds.

Clones: a number of copies of a DNA fragment that have been replicated by a phage or plasmid in a host bacterial cell.

Colloid: Microscopic particles suspended in a liquid medium, usually between one nanometer and one micrometer in size.

Cometabolism: Biodegradation of a substance (pollutant) by an organism that uses some other compound for growth and energy.

Commensalism: An interactive association between organisms from different species living in close proximity to one another in which one population benefits from the association while the other is not affected.

Complex: A type of compound in which a central metal ion is surrounded by a number of ions or molecules, called ligands, that can also exist separately, also known as a coordination compound. A chelate is a type of complex.

Complexing agent: A dissolved ligand that binds with a simple charged or uncharged molecular species in a liquid solution to form a complex, or coordination compound.

Conjugation: The process by which genetic material is transferred from one microorganism to another, involving a physical connection between the two cells.

Consortium: A group of organisms that interact within a given environment, generally resulting in combined metabolic activities.

Contaminant: Harmful or hazardous matter introduced into the environment.

Coprecipitation: The incorporation of elements into other compounds, such as metal oxide minerals, as they precipitate from solution.

Covalent bond: A nonionic chemical bond formed between atoms by the sharing of electrons.

Cytochrome: A heme-containing protein that is involved in oxidation–reduction reactions.

Cytoplasm: Cellular contents inside the cytoplasmic membrane, minus the nucleus, which include the membrane-bound organelles (such as mitochondria or chloroplasts) and cytoplasmic fluid.

Cytoplasmic membrane: The permeable membrane surrounding the cell's cytoplasm; also called cell membrane.

Denitrification: Conversion by microorganisms of nitrate or nitrite to more reduced states, ending in nitrogen gas under anaerobic conditions.

Dense non-aqueous phase liquid (DNAPL): Liquid contaminants that are relatively insoluble and heavier than water.

Deoxyribonucleic acid (DNA): The molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases adenine (A), guanine (G), cytosine (C), and thymine (T). In nature, base pairs form only between A's and T's and between G's and C's. Therefore, the base sequence of each single strand can be deduced from that of its partner.

Diffusion: The natural tendency of molecules to move out of areas of high concentration into areas of low concentration until a solution or gas has a uniform concentration of the molecules.

Dispersion: The distribution of a solute throughout a solvent, as in sugar in water.

Dissimilative metabolism: The use of an inorganic compound (such as nitrate) as an electron acceptor in energy metabolism; that is, the compound is not used to satisfy nutritional needs.

Ecology: The study of interrelationships between organisms and their environment.

Eh: Oxidation–reduction potential; the relative susceptibility of a substrate to oxidation or reduction.

Electron: A stable atomic particle that has a negative charge.

Electron acceptor: Small inorganic or organic compound that is reduced in a metabolic redox reaction.

Electron donor: Small inorganic or organic compound that is oxidized in a metabolic redox reaction.

Element (chemical element): Any substance that cannot be decomposed into simpler substances by ordinary chemical processes.

Enzyme: A complex protein that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.

Eukarya: The phylogenic domain consisting of one-celled and multicelled organisms called eukaryotes that maintain their genome within a defined nucleus.

Ex situ: In a position or location other than the natural or original one.

Exergonic reaction: A chemical reaction that releases energy.

Facultative: Used to indicate that an environmental factor is optional. A facultative anaerobe normally grows in the presence of oxygen, but in its absence can grow without oxygen.

Fermentation: Catabolic reaction in which organic compounds serve as both primary electron donor (substrate) and terminal electron acceptor, and in which ATP is produced by substrate-level phosphorylation.

Fission: A nuclear reaction in which an atomic nucleus, especially a heavy nucleus such as an isotope of uranium, splits into fragments, usually two fragments of comparable mass, with the evolution of from 100 million to several hundred million electron volts of energy.

Functional group: A characteristic reactive unit of a chemical compound, especially in organic chemistry.

Fungi: Spore-producing eukaryotic organisms that lack chlorophyll; examples include molds, rusts, mildews, smuts, mushrooms, and yeasts.

Gene: A sequence of nucleotides that specifies a particular polypeptide chain or RNA sequence or that regulates the expression of other genes.

Gene probe: A small molecule of single-stranded RNA or DNA with a known sequence of nucleotides used to detect a gene with a complementary nucleotide sequence.

Genetic engineering: The use of in vitro techniques in the isolation, manipulation, recombination, and expression of DNA, which includes the reintroduction of the affected genes into cells of the same or different species.

Genome: The sum of all chromosomal genes in a cell.

Genomics: The scientific study of the genome.

Genotype: All or part of the genetic constitution of an individual or group.

Ground water: Water found beneath the Earth's surface that fills pores between materials, such as sand, soil, or gravel; supplies wells and springs.

Half-life: The time required for half of the atoms of a radioactive substance to disintegrate.

Heavy metals: Metallic elements with high molecular weights. Such metals are often residual in the environment, exhibit biological accumulation, and are generally toxic in low concentrations. Examples include chromium, mercury, and lead.

Heterogeneous: Consisting of diverse or dissimilar constituents.

Heterotroph: An organism that uses an organic source of carbon.

Humic: Relating to humus, which is a material resulting from partial decomposition of plant or animal matter that forms the organic portion of soil.

Hydraulic gradient: Slope or elevation difference that influences ground water velocity.

Hydrocarbons: Any of a large class of organic compounds containing only carbon and hydrogen.

Hydrolysis: The splitting of a bond by a reaction with water, specifically the addition of the hydrogen cation and the hydroxide anion of water.

Immobilization: The precipitation or binding of a substance so that it is no longer able to circulate freely.

In situ: In the original position or place.

Indigenous: Native to a particular habitat; naturally occurring.

Inoculant: Material introduced into another medium or environment; in bioremediation, a microorganism. Also inoculum.

Inorganic compounds: Chemicals that do not contain carbon; for example, metals are inorganic.

Insoluble: Not readily dissolved in a liquid.

Intrinsic bioremediation: Bioremediation at a given site as a function of the naturally occurring microbial communities and environmental conditions. A key component of natural attenuation.

Ion: An atom or group of atoms that carries a positive or negative electric charge as a result of having lost or gained one or more electrons; a charged subatomic particle (as a free electron).

Ion exchange: A reversible reaction in which ions are interchanged. This phenomenon is common in soils.

Ionic bond: A chemical bond formed between oppositely charged species because of their mutual electrostatic attraction.

Isotope: Any of two or more species of atoms of a chemical element with the same atomic number (number of protons) and nearly identical chemical behavior but with a different number of neutrons, hence a different atomic weight.

Landfill: A site where solid waste is dumped; some landfills are specially designed to serve as repositories for hazardous solid waste.

Leaching: The process of separating the soluble components from some material by percolation.

Ligand: A group, ion, or molecule coordinated to a central atom or molecule in a complex.

Light non-aqueous phase liquid (LNAPL): Liquid contaminants that are relatively insoluble and lighter than water.

Lipid: Water-insoluble organic molecule important in the structure of the cell membrane and (in some organisms) the cell wall.

Long-term stewardship: The physical controls, institutions, information, and other mechanisms needed to ensure protection of people and the environment.

Metabolic pathway: A sequence of enzymatically catalyzed chemical reactions in cellular metabolism.

Metabolism: All biochemical reactions in a cell, both anabolic and catabolic.

Methanogen: Microorganism that produces methane.

Methanogenesis: Microbial production of methane (CH_4) through the reduction of CO_2. This reduction is coupled to oxidation of hydrogen, or certain organic compounds.

Methanotroph: Aerobic microorganism that can oxidize methane as a sole source of carbon.

Methylation: Substitution of a methyl group for a hydrogen atom.

Microarray: High-density, miniaturized, nucleic acid hybridization technologies.

Microbiology: A branch of biology dealing with microscopic forms of life (bacteria, archaea, protozoa, algae, viruses, and fungi).

Microorganism: Any organism of microscopic or ultramicroscopic size.

Mineralization: The complete breakdown of organic materials by microorganisms into inorganic materials such as carbon dioxide and water.

Mitochondria: Cellular organelles of most eukaryotes found outside the nucleus that produce energy for the cell through aerobic respiration.

Mixed Waste: Waste that contains both radioactive and (chemically) hazardous components.

Molecule: The smallest particle of a substance that retains all the properties of the substance and is composed of one or more atoms.

Mutualism: A type of symbiosis whereby organisms from different species live in close proximity to one another, in which all organisms involved benefit from the relationship.

Mycoremediation: The use of fungi to reclaim soils, sediments, and water that have been polluted by substances hazardous to humans and the environment. A form of bioremediation.

Natural attenuation: Allowing a variety of natural physical, chemical, and biological processes to reduce the amount, toxicity, mobility, and concentration of contaminants in the environment. These processes include biological degradation, dilution, sorption to soil or aquifer particles, volatilization to the atmosphere, and chemical reactions with natural materials.

Nitrate reduction: The reduction of nitrate to reduced forms of nitrogen; under anaerobic and microaerophilic conditions, some bacteria may use nitrate as a terminal electron acceptor for respiratory metabolism.

Nitrification: The oxidation of ammonia to nitrite and then nitrate by microorganisms. Occurs under aerobic conditions.

Nucleotide: The combination of a purine or pyrimidine base with a sugar and phosphoric acid. The basic structural unit of nucleic acid.

Obligate: Used to indicate that an environmental factor is required for growth. An obligate aerobe always requires oxygen for growth.

Organic compounds: Chemical compounds that contain carbon and hydrogen, elements usually associated with life processes.

Oxidant: A molecule or atom that accepts electrons in an oxidation–reduction reaction.

Oxidation–reduction reaction: Coupled reactions in which one compound becomes oxidized (releases electrons) while another becomes reduced, gaining the electrons released.

Percolation: Gravity flow of ground water through the pore spaces in rock or soil, usually from the unsaturated zone to the saturated zone; passing of a solvent through a permeable substance.

Permeable reactive barriers (PRBs): In situ treatment zones that are engineered down-gradient from a contaminant plume. As ground water passes through the treatment zone, contaminants are adsorbed, reduced and precipitated, biodegraded, biotransformed, or chemically degraded.

Permeability: The capacity of a porous medium to transmit a fluid.

pH: A measure of acidity and alkalinity of a solution that is a number on a scale from 0 to 14. A value of 7 represents neutrality, lower numbers indicate increasing acidity, and higher numbers increasing alkalinity. This change in acidity or alkalinity is the negative logarithm of the effective hydrogen-ion concentration or hydrogen-ion activity in gram equivalents per liter of the solution. Thus, each unit of change (e.g., from 7 to 6) represents a tenfold change in acidity or alkalinity.

Phenotype: The observable properties of an organism; the manifestation of gene expression in that organism.

Phototroph: An organism that gets its energy from light.

Phytoremediation: The use of plants to treat soils, sediments, and water that have been polluted by substances

hazardous to humans and the environment. A form of bioremediation.

Plasmids: a self-replicating linear or circular molecule of DNA distinct from chromosomal DNA. Some plasmids carry genes important to bioremediation.

Plume: An elongated body of fluid, usually mobile and varying in shape. Used to define the contaminated areas of an environment.

Porosity: The volume of aquifer material that is not occupied by solids.

Precipitation: The process whereby a solid settles out of a solution.

Prokaryote: One-celled microorganism whose genome is not contained within a nucleus. Comprising the two domains Bacteria and Archaea.

Proteomics: The study of the complete complement of proteins in a cell.

Protein: A large molecule composed of one or more chains of amino acids in a specific order joined by peptide bonds, containing the elements carbon, hydrogen, nitrogen, oxygen, usually sulfur, and sometimes other elements such as phosphorus and iron. Many essential biological compounds are composed of proteins, including enzymes.

Proton: Positive hydrogen ion.

Proton motive force: An energized state of a membrane created by expulsion of protons through the action of an electron transport chain.

Radioactivity: Spontaneous emission by radionuclides of energetic particles through the disintegration of their atomic nuclei; the rays emitted.

Radioisotope: An isotope of an element that has an unstable nucleus; it tries to stabilize itself by giving off radioactive particles and undergoes spontaneous decay.

Radionuclide: Radioisotope.

Reactant: A substance that enters into and is altered in the course of a chemical reaction.

Reaction: A process in which one or more substances are changed chemically into one or more different substances.

Recalcitrant: Resistant to degradation/transformation.

Redox reaction: Oxidation–reduction reaction in which electrons are transferred between two or more compounds.

Reductant: A molecule or atom that donates an electron in an oxidation–reduction reaction.

Reduction potential: The inherent tendency of a compound to act as an electron donor or an electron acceptor; measured in volts.

Respiration: A series of catabolic redox reactions that produce ATP in which organic or inorganic compounds are primary electron donors and organic or inorganic compounds are terminal electron acceptors.

Rhizosphere: Soil that surrounds and is influenced by the roots of a plant.

Ribonucleic acid (RNA): A linear polymer of nucleotides containing ribose and uracil as structural components. RNA plays an important role in protein synthesis and cell metabolism.

Saturated zone: An underground geologic layer in which all pores and fractures are filled with water.

Sediment: Material in suspension in water or deposited from suspension or precipitation.

Siderophore: Chelator that solubilizes metals, such as iron hydroxides, making them available for uptake by microorganisms.

Solubility: The relative capacity of a substance to serve as a solute, usually in reference to water as the solvent.

Soluble: Able to be dissolved; to pass into solution.

Solute: Any material that is dissolved in another, such as salt dissolved in water.

Solution: A homogeneous mixture of a solute in a solvent. When a solute is dissolved in a solvent, the solute molecules are separated from one another and dispersed throughout the liquid medium.

Solvent: Any material that dissolves another, such as water dissolving salt.

Sorption: The process of being taken up or held by either adsorption or absorption.

Substrate: The substance acted upon by an enzyme.

Substrate-level phos phorylation: Synthesis of ATP through the transfer of phosphate from an activated (usually) organic substrate to ADP. Occurs during fermentation.

Subsurface: The geologic zone below the surface of the Earth.

Superfund Trust Fund: A public trust fund created with passage of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) in 1980 to be used to help pay for the cleanup of abandoned hazardous waste sites. This law, nicknamed Superfund, provides the authority through which the federal government can compel people or companies to clean up hazardous waste sites they are responsible for creating. Superfund also assists with the cleanup of inactive and abandoned hazardous waste sites or accidentally spilled or illegally dumped hazardous materials.

Surfactant: A natural or synthetic chemical that promotes the wetting, solubilization, and emulsification of various types of organic chemicals. Detergents are surfactants.

Symbiosis: A type of interaction where individuals of one species live in intimate association with those of another.

Syntrophy: A form of mutualism in which the members of two species are nutritionally dependent on one another.

Transport: Conveyance of solutes and particles in flow systems.

Transuranic: Relating to or being an element with an atomic number greater than that of uranium (92).

Unsaturated zone: An underground geologic layer in which pores and fractures are filled with a combination of air and water.

Vadose zone: The unsaturated zone above the water table. Also known as zone of aeration.

Valence: The property of an element that determines the number of other atoms with which an atom of the element can combine.

Volatile organic compounds (VOCs): Organic compounds that evaporate at room temperature.

Volatilization: Vaporization.

Water table: The upper limit of a geologic layer wholly saturated with water.

Xenobiotics: A man-made substance; one that is not formed by natural biosynthetic processes.

Acronyms

ANN: Artificial Neural Network
ATP: Adenosine triphosphate
BASIC: Bioremediation and Its Societal Implications and Concerns program
DNA: Deoxyribonucleic acid
DNAPL: Dense non-aqueous phase liquid
DOE: United States Department of Energy
EDTA: Ethylenediaminetetraacetic acid
EM: DOE's Office of Environmental Management
EXAFS: Extended x-ray fine structure
FAME: Fatty acid methylester
FBR: Fluidized bed reactor
FRC: Field Research Center
FTICR: Fourier Transform Ion Cycle Resonance
GEM: Genetically engineered microorganism
HLW: High-level radioactive waste
LNAPL: Light non-aqueous phase liquid
MV: Membrane vesicle
NABIR: Natural and Accelerated Bioremediation Research program
NAPL: Non-aqueous phase liquid
NOM: Natural organic matter
NTA: Nitrilotriacetic acid
ORF: Open reading frame
PAH: Polycyclic aromatic hydrocarbon
PLFA: Polar lipid fatty acids
PCB: Polychlorinated biphenyl
PCR: Polymerase chain reaction
PRB: Permeable Reactive Barrier
RNA: Ribonucleic acid
rRNA: Ribosomal RNA
STCGs: Site Technology Coordination Groups
UMTRA: Uranium Mill Tailings Remedial Action
TCE: Trichloroethylene
TEM: Transmission electron micrograph
T-RFLP: Terminal Restriction Fragment Length Polymorphism
TRLFS: Time-resolved laser fluorescence spectroscopy
VOC: Volatile organic compound
XANES: X-ray absorption near-edge structure
XRD: X-ray diffraction

References

General

Alexander, M., and R.C. Loehr. 1992. Bioremediation review. *Science* 258(5084):874.

Barkay, T., and J. Schaefer. 2001. Metal and radionuclide bioremediation: issues, considerations and potentials. *Current Opinion In Microbiology* 4(3):318–323.

Bitton, G., and C.P. Gerba. 1984. *Ground Water Pollution Microbiology*. Wiley, New York. 377 pp.

Chapelle, F. 2000. *Ground Water Microbiology and Geochemistry*. John Wiley & Sons, New York. 477 pp.

Flathman, P.E., D.E. Jerger, and J.H. Exner. 1994. *Bioremediation Field Experience*. CRC Press, Boca Raton, FL. 548 pp.

Fredrickson, J.K and M. Fletcher. 2001. *Subsurface Microbial Ecology and Biogeochemistry*, John Wiley & Sons, New York, 352 pp.

Hurst, C.J. et al. (eds.). 2001. *Manual of Environmental Microbiology*, 2nd edition. ASM Press, Washington, D.C. 1158 pp.

Lengeler, J.,G. Drews, and H. Schlegel. 1998. *Biology of the Prokaryotes*. Blackwell Science, Oxford. 984 pp.

Lovley, D.R. 2001. Bioremediation — anaerobes to the rescue. *Science* 293(5534):1444–1446.

Lovley, D. 2000. *Environmental Microbe-Metal Interactions*. ASM Press, Washington, D.C., 395 pp.

Nealson, K.H., A. Belz, and B. McKee. 2002. Breathing metals as a way of life: geobiology in action. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 81:215–222.

Norris, R.D. et al. 1994. *Handbook of Bioremediation*. CRC Press, Boca Raton, FL. 257 pp.

Perry, J.J., J.T. Staley, and S. Lory. 2002. *Microbial Life*. Sinauer Associates, Publishers. Sunderland, MA. 811 pp.

Philip, J.C., R.M. Atlas, et al. 2000. Bioremediation. *Electronic Encyclopaedia of the Life Sciences*. Nature Publishing Group, London.

Schlesinger, W. H. 1997. *Biogeochemistry: An Analysis of Global Change*. Academic Press, New York. 588 pp.

Section I. The Problem: Metals and Radionuclides at DOE Sites

Environmental Management Research and Development Program Plan. 1998. U.S. Department of Energy, Washington, D.C.

Linking Legacies Report. 1997. DOE/EM-319, U.S. Department of Energy, Washington, D.C.

Environmental Quality: Long Term Stewardship. 2002. U.S. Department of Energy, Washington, D.C.

Nuclear Age Timeline Report. 1999. U.S. Department of Energy, Washington, D.C. Online report: http://www.em.doe.gov/timeline/index.html

Riley, R.G. et al. 1992. *Chemical Contaminants on DOE Lands and Selection of Contaminant Mixtures for Subsurface Research*. DOE/ER-0547T, U.S. Department of Energy, Washington, D.C.

Status Report on Paths to Closure. 2000. U.S. Department of Energy, Washington, D.C. Online report: http://www.em.doe.gov/closure/fy2000/index.html.

Section II. What is Bioremediation?

Alexander, M. 1999. *Biodegradation and Bioremediation*. Academic Press, San Diego, 453 pp.

Hazen, T.C. 1997. Bioremediation. In *Microbiology of the Deep Terrestrial Subsurface*. P. Amy and D. Haldeman (eds.), CRC Press, Boca Raton, FL, pp. 247–266.

National Research Council. 2000. *Natural Attenuation for Ground Water Remediation*. National Academy Press, 292 pp.

Section III. Metals and Radionuclides Found at Contaminated Sites

Bethke, C.M., *Geochemical Reaction Modeling: Concepts and Applications*. Oxford University Press, 1996, 397 pp.

Burns, P.C. and R. Fitch. 1999. *Uranium: Mineralogy, Geochemistry and the Environment. Reviews in Mineralogy, Volume 8*. Mineralogical Society of America, 679 pp.

Choppin, G.R., J.-O. Liljenzin, and J. Rydberg.

2001. *Radiochemistry and Nuclear Chemistry*, 3rd edition. Butterworth and Heinemann (eds.), 728 pp (available for reference on line at http://book.nc.chalmers.se/).

Cleveland, J.M. 1979. *The Chemistry of Plutonium*. American Nuclear Society, La Grange Park, IL, 653 pp.

Rai, D. et al. 2001. Thermodynamic model for the solubility of PuO_2(am) in the aqueous Na^+-H^+-OH^--Cl^--H_2O-ethylenediaminetetraacetate system. *Radiochim. Acta* 89:67–74.

Stumm, W. 1992. *Chemistry of the Solid-Water Interface*. John Wiley & Sons, Inc., 428 pp.

Section IV. A Look at Microbial Metabolism

Brim et al. 2000. Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nature Biotechnology* 18:85–90.

Burgos, W.D. et al .2002. Theoretical and experimental considerations related to reaction-based modeling: A case study using iron(III) oxide bioreduction. *Geomicrobiology Journal* 19:253–287.

Childers, S.E., S. Ciufo, and D.R. Lovley. 2002. *Geobacter metallireducens* access Fe(III) oxides by chemotaxis. *Nature* 416:767–769.

Kostka , J.E. et al. 2002. Growth of iron(III)-reducing bacteria on clay minerals as the sole electron acceptor and comparison of growth yields on a variety of oxidized iron forms. *Applied Environmental Microbiol*ogy 68:6256–6262.

Hedrick, D.B. et al. 2000. Measuring soil microbial community diversity using polar lipid fatty acid and denaturing gradient gel electrophoresis data. *Journal of Microbiological Methods* 41:235–248.

Lovley, D. 2002. Dissimilatory metal reduction: from early life to bioremediation. *ASM News* 68: 231–237.

Lovley, D.R. and E.L. Blunt-Harris. 1999. Role of humics-bound iron as an electron transfer agent in dissimilatory Fe(III) reduction. *Applied and Environmental Microbiology* 65:4252–4254.

Marsh, T.L. 1999. Terminal restriction fragment length polymorphism(T-RFLP). An emerging method for characterizing diversity among homologous populations of amplification products. *Current Opinions in Microbiology* 2:323–327.

Oxtoby, D. W., N. H. Nachtrieb, and W. A. Freeman. 1994. *Chemistry, Science of Change*, 2nd edition. Saunders College Publishing, Philadelphia, PA.

Newman, D.K., and R. Kolter. 2000. A role for excreted quinones in extracellular electron transfer. *Nature* 405:94–97.

Sani, R.K. et al. 2002. Dissimilatory reduction of Cr(VI), Fe(III), and U(VI) by *Cellulomonas* isolates. *Applied Microbiology and Biotechnology* 60: 192–199.

Schwarzenbach, R.P., P.M. Gschwend, and D.M. Imboden. 1993. *Environmental Organic Chemistry*. John Wiley & Sons, Inc. 681 pp.

Stumm, W. and J.J. Morgan, 1996. Aquatic Chemistry, 3rd edition. Wiley and Sons, Inc., NY.

Tinoco, Jr., I., K. Sauer, J. C. Wang. 1985. *Physical Chemistry: Principles and Applications in Biological Sciences*, 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, NJ.

White, O. et al. 1999. Sequencing and functional analysis of the *Deinococcus radiodurans* genome. *Science* 286:1571–1577.

Wu, L.Y., et al. 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. *Applied and Environmental Microbiology* 67:5780–5790.

Section V. Microbial Processes Affecting Bioremediation of Metals and Radionuclides

Bolton, H., L. Xun, and D. C. Girvin. 2000. Biodegradation of synthetic chelating agents. In *Environmental Microbe-Metal Interactions*. D. Lovley (ed.). ASM Press, Washington, D.C. pp. 363–383.

Daly, M.J., 2000. Engineering radiation-resistant bacteria for environmental biotechnology. *Current Opinions in Biotechnology* 11:280–285.

Fendorf, S., B.W. Wielinga, and C.M. Hansel. 2000. Chromium transformations in natural environments: The role of biological and abiological processes in chromium(VI) reduction. *International Geological Reviews* 42:691–701.

Francis, A.J., and C.J. Dodge. 1998. Remediation of soils and wastes contaminated with uranium and toxic metals. *Environmental Science & Technology* 32:3993–3998.

Fredrickson, J.K., et al. 2000. Reduction of Fe(III), Cr(VI), U(VI), and Tc(VII) by *Deinococcus radiodurans*. *Applied and Environmental Microbiology* 66:2006–2011.

Fredrickson, J.K., et al. Influence of Mn oxides on the reduction of uranium(VI) by the metal-reducing bacterium *Shewanella putrefaciens*. *Geochemica Cosmochimica Acta* 66:3247–3262.

John, S.G., et al. 2001. Siderophore mediated plutonium accumulation by *Microbacterium flavescens* (JG-9). *Environmental Science & Technology* 35:2942–2948.

Labrenz, M., et al. 2000. Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. *Science* 290:1744–1747.

Lack, J.G. et al. 2002. Immobilization of radionuclides and heavy metals through anaerobic bio-oxidation of Fe(II) by *Dechlorosoma suillum*. *Microbial Ecology* 43: 424–431.

Lloyd, J.R. and D.R. Lovley. 2001. Microbial detoxification of heavy metals and radionuclides. *Current Opinions in Biotechnology* 12:248–253.

Neu, M.P. et al. 2000. Siderophore-mediated chemistry and microbial uptake of plutonium. *Los Alamos Science* 26(2):416-417.

Neu, M.P., J.H. Matonic, C.E. Ruggiero, B.L. Scott, and Angew. 2000. The first structural characterization of plutonium(IV) complexed by a siderophore: Single crystal structure of *Pu Desferrioxamine E. Angewandte Chemie International Edition* 39(8):1442–1444.

Payne, R.B. et al. 2002. Uranium reduction by *Desulfovibrio desulfuricans* strain G20 and a cytochrome c_3 mutant. *Applied and Environmental Microbiology* 68:3129–3132.

Section VI. Field Research on Bioremediation of Metals and Radionuclides

Chang, Y.-J. et al. 2001. Diversity and characterization of sulfate reducing bacteria in ground water at a uranium mill tailings site. *Applied Environmental Microbiology* 67:3149–3160.

Holmes, D.E., K.T. Finneran and D.R. Lovley. 2002. Enrichment of *Geobacteraceae* associated with stimulation of dissimilatory metal reduction in uranium contaminated aquifer sediments. *Applied Environmental Microbiology* 68:2300–2306.

Istok, J.D. et al. 1997. Single-well, "push-pull" test for in situ determination of microbial activities. *Ground Water* 35:619–631.

Senko, J. M., et al. 2002. In situ evidence for uranium immobilization and remobilization. *Environmental Science & Technology* 36:1491–1496.

Tokunaga, T.K., et al. 2001. Chromium diffusion and reduction in soil aggregates. *Environmental Science & Technology* 35:3169–3174.

Bioremediation Web Sites

American Society for Microbiology
http://www.asm.org

Biocatalysis/Biodegradation Database
http://umbbd.ahc.umn.edu/

Environmental Management Science Program (EMSP)
http://emsp.em.doe.gov

Environmental Molecular Science Laboratory
http://www.emsl.pnl.gov

Environmental Protection Agency-Bioremediation Documents
http://www.epa.gov/ORD/WebPubs/biorem/

Environmental Protection Agency-Technology Innovation Office of Science
http://clu-in.com/

Genomes to Life Program
http://www.GenomestoLife.org

Hanford Ground Water/Vadose Zone Integration Project
http://www.bhi-erc.com/projects/vadose/

International Society for Microbial Ecology
http://www.microbes.org

Joint Genome Institute
http://www.jgi.org

The Microbe Zoo
http://commtechlab.msu.edu/sites/dlc-me/zoo/

Microbial Genomics Gateway
http://www.microbialgenome.org

Microscopy Primer
http://micro.magnet.fsu.edu/primer/anatomy/anatomy.html

National Center for Genome Resources
http://www.ncgr.org

Natural and Accelerated Bioremediation Research Program
http://www.lbl.gov/NABIR

NABIR Field Research Center at Oak Ridge National Laboratory
http://www.esd.ornl.gov/nabirfrc/

Office of Biological and Environmental Research
http://www.sc.doe.gov/production/ober/ober_top.html

Office of Environmental Management
http://ww.em.doe.gov/index4.html

Periodic Table of Elements
http://www.webelements.com/webelements/

Ribosomal Database Project
http://www.cme.msu.edu/RDP

Savannah River Ecology Laboratory
http://www.uga.edu/~srel

Strategic Environmental Research and Development Program (SERDP)
http://www.serdp.org

Superfund Basic Research Program
http://www.niehs.nih.gov/sbrp/home.htm

The Institute for Genomic Research (TIGR)
http://www.tigr.org

U.S. Department of Agriculture — Bioremediation Site
http://www.nal.usda.gov/bic/Biorem/biorem.htm

U.S. Geological Survey — Bioremediation Site
http://water.usgs.gov/wid/html/bioremed.html

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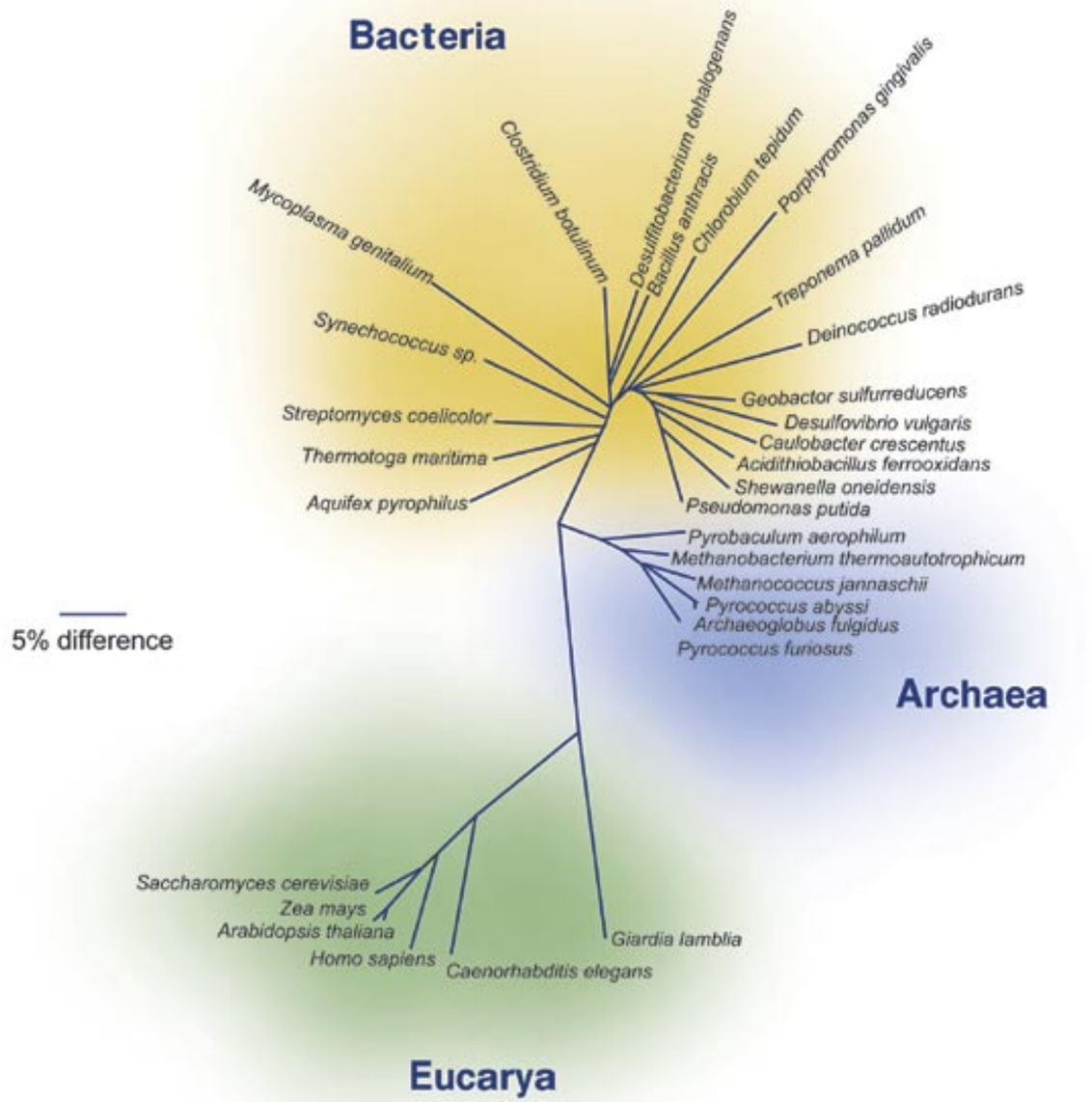

The Molecular Tree of Life. (Courtesy of James Cole, Michigan State University.)

Ernest Orlando Lawrence Berkeley National Laboratory
Earth Sciences Division
1 Cyclotron Road • Mailstop 90-1116
Berkeley, California 94720