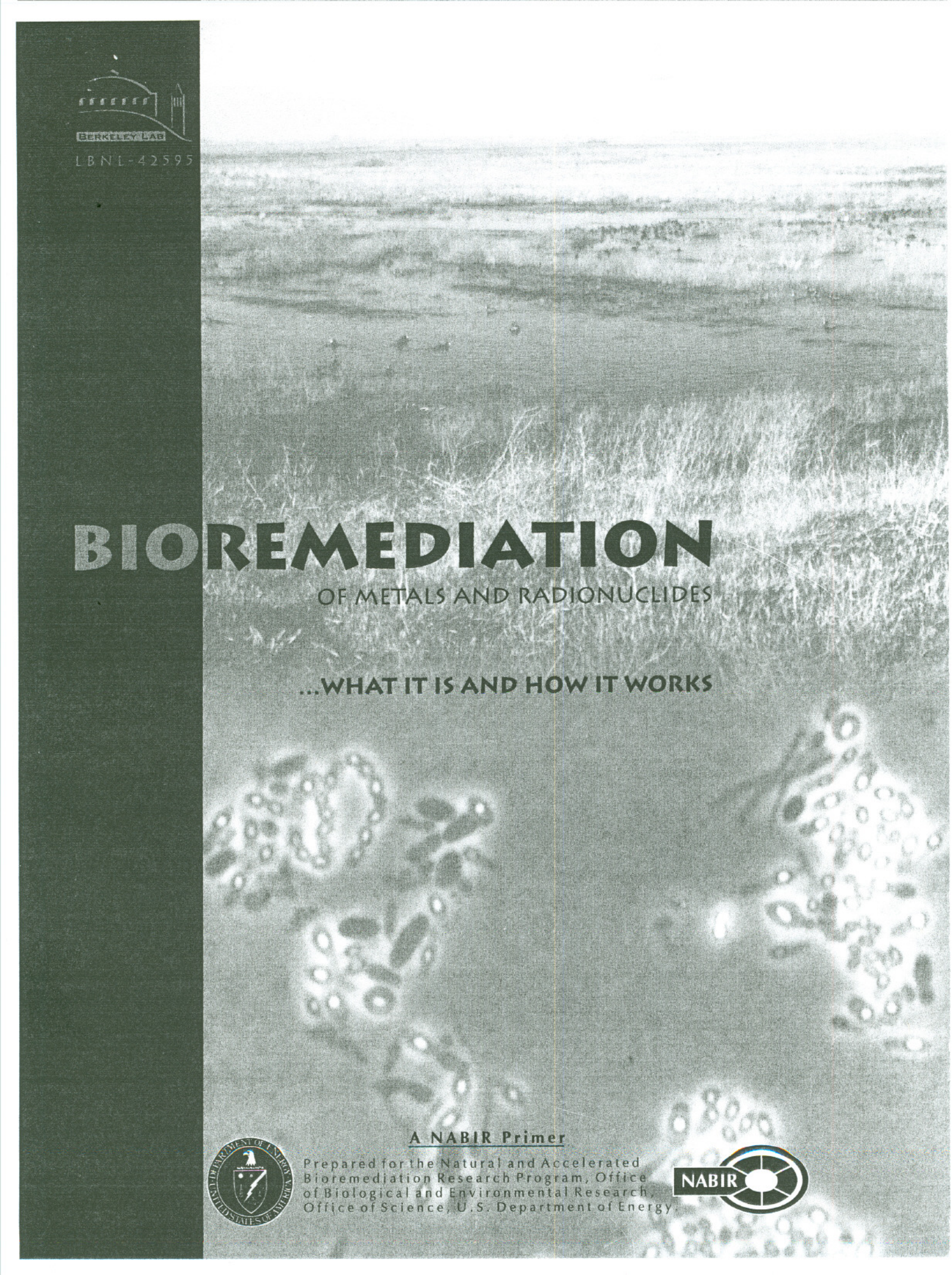

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BIOREMEDIATION

OF METALS AND RADIONUCLIDES

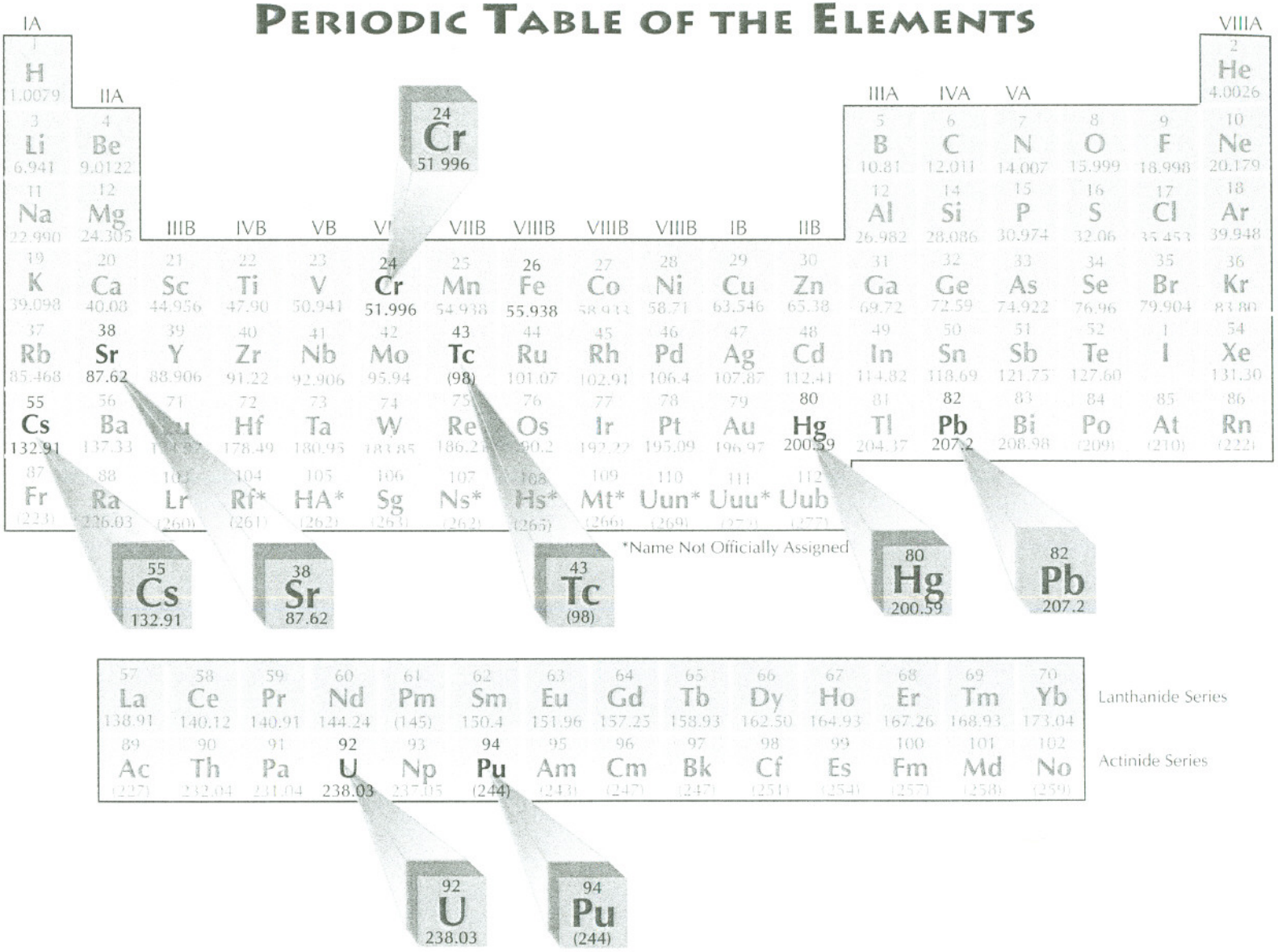
...WHAT IT IS AND HOW IT WORKS

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

NABIR

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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FOREWORD

This primer is intended for people interested in DOE environmental problems 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.[1]

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 physical environment, microbial communities, and nature of contaminant. This technology includes intrinsic bioremediation, which relies on naturally occurring processes, and accelerated bioremediation, which enhances microbial degradation or transformation through inoculation with microorganisms (bioaugmentation) or the addition of nutrients (biostimulation).

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 like 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 donors or acceptors. In some cases, the solubility of the altered species increases, increasing the mobility of the contaminant and allowing it to more easily be flushed from the environment. In other cases, the opposite will occur, and the contaminant will be immobilized in situ, e.g., precipitated into an insoluble salt in the sediment. 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 of groundwater, sediment, and soil contamination at Department of Energy facilities.

Subsurface bioremediation of metals and radionuclides at the site of contamination (in situ bioremediation), particularly of contaminants found in mixed waste, 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 a hypothetical case study of a composite DOE site with polluted groundwater containing some of the Department of Energy's most recalcitrant contaminants.

1. DOE's Office of Science has a bioremediation research program entitled Natural and Accelerated Bioremediation Research (NABIR). NABIR 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.

WHY IS IT NECESSARY TO CLEAN UP DOE SITES?

OUR NATION'S COLD WAR LEGACY

For more than 50 years the United States has used nuclear energy for both peaceful 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, 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, sediments, and the over 7,000 structures spread over 7,280 square kilometers. The Department must characterize, treat, and dispose of hazardous and radioactive waste at more than 120 sites in 36 states and territories. This includes 475 billion gallons of contaminated groundwater in 5,700 distinct plumes, 75 million cubic meters of contaminated sediments, and 3 million cubic meters of leaking waste buried in landfills, trenches, and spill areas (*Linking Legacies Report*, January 1997). The first few years of this activity, up to 1995, have mainly involved cataloging and preliminary characterization. This alone has cost the Department more than $23 billion. Budget projections for these activities just for the next 10 years exceed $60 billion. The DOE cleanup of the Cold War Legacy is the largest program of its kind ever undertaken by the United States.

OVERALL ENVIRONMENTAL RESTORATION

DOE's Office of Environmental Management (EM) has the major responsibility for this enormous clean-up effort. EM has four major objectives for its science and technology investments (*EM Research and Development Program Plan*, October 1998): (1) meet high-priority needs; (2) reduce the cost of EM's major cost centers (areas where DOE has its major cleanup investments); (3) reduce EM's technological risk; and (4) accelerate technology deployment. To meet these objectives, EM has sought the assistance of the basic research programs in DOE's Office of Biological and Environmental Research, especially the Natural and Accelerated Bioremediation Research (NABIR) program. In addition, EM has established ten Site Technology Coordination Groups (STCGs) to coordinate technology assessments at the main hazardous waste sites in the DOE complex. (See "Bioremediation Web Sites" at the end of this primer for a link to the STCG web sites.) Each STCG maintains a dynamic list of its sites' highest priority science and technology needs for effective cleanup. This list is updated annually. From this list, EM 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 and saturated zones. Improved analytical tools, in situ monitoring devices, understanding of permeability patterns, and tools to predict groundwater 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 contaminating 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 resolution of problems in several areas. Bioreactive treatment methods are needed for remediation of low to moderate concentrations of organic solvents in sediments and groundwater. Chemical treatment technologies to destroy or immobilize highly concentrated contaminant source terms (metals, radionuclides, explosive residues, and solvents) in the vadose and saturated zones are required to increase remediation rates. Finally, improved deep drilling technology is needed 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 NABIR program addresses a large number of DOE's environmental restoration needs by conducting basic research on natural and accelerated bioremediation, especially as it relates to radionuclides and metals in subsurface environments. The research being funded by the program specifically focuses on one or more components in each of the above five need areas. The necessity for basic research to focus on radionuclides and metals is further illustrated by a review of DOE contaminants by waste site and facility (Riley et al., 1992). This review of DOE chemical contaminants and mixtures for the Subsurface Science Program is one of the few comprehensive comparisons of DOE contaminants ever done. This report 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). However, the first two classes by facility (fuel and chlorinated hydrocarbons) are technologically further advanced in the development of cost-effective and efficient solutions. Therefore, remediation of radionuclides and metals currently requires greater research emphasis to support technology development.

Contaminants in groundwater at DOE facilities are also dominated by metals and radionuclides, 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 contaminant that exceeds the frequency of metal and radionuclide contamination in groundwater is chlorinated hydrocarbons, for

which there are already a large number of potential solutions.

The need for basic research to focus on metals and radionuclides is further underscored by the recognition that radionuclides are a uniquely DOE problem. Because nuclear production was carried out by the 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 subsurface mobilization and immobilization of radionuclides and metals will allow us to manipulate, stabilize, and predict long-term stability of these contaminants and their relative risk. This research will not only facilitate our overall understanding of our environment, but also potentially save DOE millions if not billions of dollars in life cycle costs of cleanup of the Cold War Legacy.

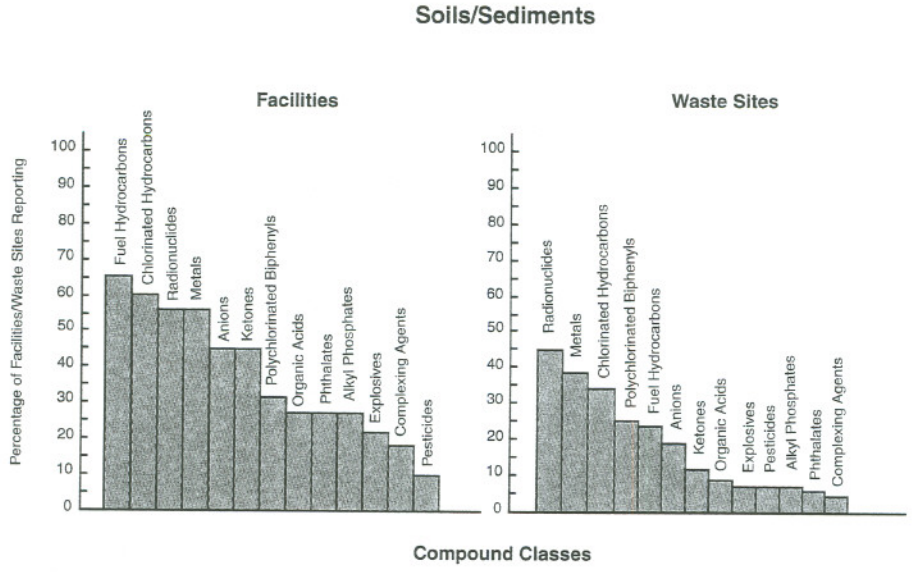

Figure 1.1. Distribution of compound classes in soils/sediments at 18 DOE facilities and 91 waste sites (Riley et al., 1992).

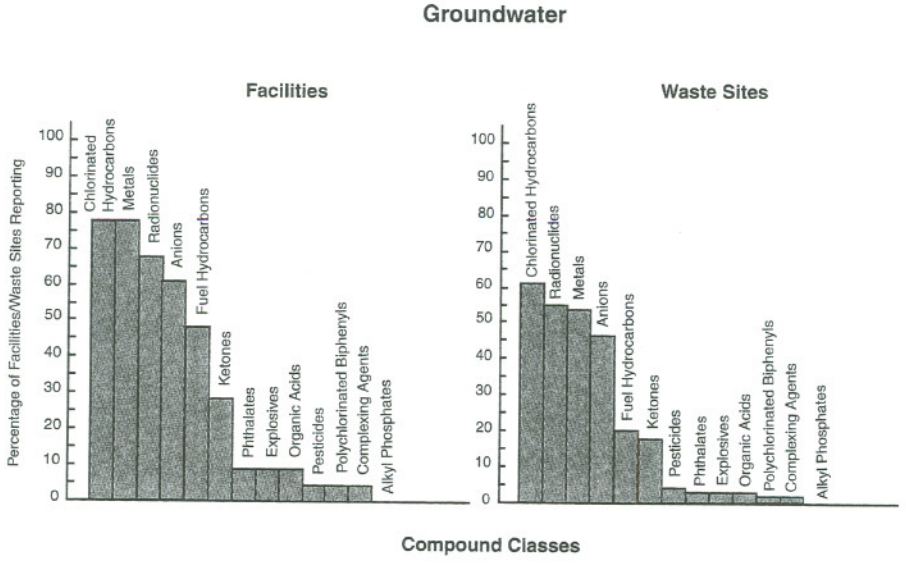

Figure 1.2. Distribution of compound classes in groundwater at 18 DOE facilities and 91 waste sites (Riley et al., 1992).

SECTION II:

WHAT IS BIOREMEDIATION?

INTRODUCTION

Bioremediation technology uses microorganisms to reduce, eliminate, or contain contaminants. It is not a new technology, however. Composting, sewage treatment, and certain types of fermentation have been practiced by humankind since the beginning of recorded history, and all of these utilize microbial processes. 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. Yet the word "bioremediation" is fairly new. Its first appearance in peer-reviewed scientific literature was in 1987 (Hazen, 1997).

The use of this technology is gaining popularity. The last ten years have seen an increase in the types of contaminants to which bioremediation is being applied, including solvents, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Now, microbial processes are beginning to be used in the clean up of radioactive and metallic contaminants, the most recalcitrant components of hazardous waste, and the two most often found components of mixed waste at DOE sites, as discussed in Section I.

This primer looks at the possibilities for in situ bioremediation of these types of contaminants.[1] Featured are eight elements that constitute some of the most prevalent metals and radionuclides found in DOE waste: cesium, chromium, lead, mercury, plutonium, uranium, strontium, and technetium. All are metallic elements and very toxic. In addition, cesium, plutonium, strontium, technetium, and uranium are extremely radioactive. (See the inside front cover for a periodic table that highlights these elements.) In situ bioremediation of these contaminants, particularly in contaminant mixtures, is not yet in widespread use. However, successful in situ applications of bioremediation to petroleum products and chlorinated solvents are a resource from which the scientific community can draw. 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.[2]

Many remediation technologies exist to treat hazardous waste. One of the most common has been pump and treat (extraction and then treatment by various processes). Pump and treat is often applied in the remediation of industrial solvents such as trichloroethylene (TCE), which is used to degrease metals (including nuclear target elements and computer components), dry-clean clothes, and even decaffeinate coffee.

Extraction processes do have some major disadvantages. Subsurface sediment and rock formations are heterogeneous. This lack of uniformity can cause uneven flow patterns. So it can take a long time to flush contamination out of areas where water flow is slow. Many contaminants also tend to adsorb (stick) to mineral

1. This contamination often exists in the form of contaminant plumes. See the feature on page 14 for more information on how the subsurface is structured and how these plumes move.

2. Although organic components are a part of mixed waste at DOE sites, they are not the focus of this primer. However, certain organic compounds play a central role in determining metal and radionuclide bioremediation strategy. The synthetic chelators EDTA (ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid) were commonly used as cleaning agents during industrial processing of nuclear fuels at DOE and have formed stable, soluble complexes with certain heavy metals in the subsurface.

surfaces of clays or to organic materials. This can slow extraction, and it often takes 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 and the public.

Bioremediation is an alternative to traditional remediating technologies, such as landfilling or incineration. It 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 organic substances by microorganisms into smaller organic or inorganic components. Mineralization is the complete biodegradation of an organic contaminant into inorganic constituents such as carbon dioxide and water. Under anaerobic conditions, the ultimate product of biodegradation may be methane. This complete degradation of a compound is the end result of numerous biodegradation steps. These transforming and degrading processes occur as a result of microorganisms using the contaminants as a source of nutrients or energy, changing them through various metabolic reactions.

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.[3] In some cases, the solubility of the altered species increases, increasing the mobility of the contaminant and allowing it to more easily be flushed from the environment. In other cases, the opposite will occur, and the contaminant will be immobilized in situ, thus reducing the risk to humans and the environment. Both kinds of transformations present opportunities for bioremediation of metals and radionuclides — either to lock them in place or to accelerate their removal.

Although bacteria are usually the agents in most types of bioremediation, fungi and algae also can transform and degrade contaminants. Bioremediation depends on the presence of the appropriate microorganisms in the correct amounts and combinations and on the appropriate environmental conditions. Optimum environments for growth of microbes typically consist of temperatures ranging between 15 and 45°C;[4] pH values between 5.5 and 8.5; and nutrient ratios (C:N:P) of 120:10:1. Atmospheric composition and water content may also influence microbial growth and activity. In addition, the contaminants must be in close enough proximity to the microbes and in a form that the microbes can utilize.

Which Bioremediation Technology Should be Used?

Webster's Dictionary defines in situ as "in place; in the natural or original position or place." In situ bioremediation refers to below-ground methods applied at the site of contamination. Webster's defines ex situ as "in a position or location other than the natural or original one," but this usually refers to above-ground bioremediation, where the sediment or water has been extracted from the subsuface.

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; they are easier to contain and control. However, in situ bioremediation has several advantages over ex situ techniques. It offers a way of treating contaminants that are widely dispersed in the environment, present in dilute concentrations, or are otherwise inaccessible. It is more

3. Microorganisms can do much more than alter oxidation state. They are also capable of influencing contaminant behavior in other ways. Examples include changing the acidity of the system in the immediate vicinity of the contaminant and alteration of the form of organic compounds that influence radionuclide and metal mobility. Although important, these factors are not the main focus of this primer.

4. Although recently it has been discovered that petroleum bioremediation in the Arctic and Antarctic can occur at nearly the same rates at near zero degree temperatures (°C) as are seen in more temperate climates.

cost effective than ex situ techniques because no pumping or excavation is required. Also, in situ bioremediation may be less hazardous, as there is no exposure to the contaminant during treatment. This is a consideration because of the mixing of metals and radionuclides with organic contaminants at DOE sites. This mixing has resulted in modification of the contaminants' transport and toxicity properties, which often imposes an increased health risk.

Next is a brief overview of several existing bioremediation strategies. As Figure 2.1 demonstrates, these methodologies are not mutually exclusive and, depending on the type of contaminant problem, can be used in combination with one another and/or with more traditional remediation techniques.

Biostimulation and Bioaugmentation

These two bioremediation techniques can be used together or separately. They can occur either above ground (in stirred tanks called bioreactors) or below ground. Biostimulation is the addition of nutrients, oxygen, or other electron donors and acceptors to increase the number or activity of naturally occurring microorganisms available for bioremediation. These components can be added in either liquid (soil washing) or gas (soil venting) form. Biosparging is a type of soil venting where air or other gases are injected below the ground into saturated sediments.

All microorganisms need carbon. Carbon usually comes from an organic source (e.g., glucose or methane), but also can be provided in dissolved inorganic forms such as carbon dioxide (CO_2). Sometimes the contaminant is a carbon source, as in the case of gasoline contaminants such as benzene, toluene, and xylene. Waste products from plants and other microorganisms also can provide carbon. Some of the other most common microbial nutrients are nitrogen, phosphorus, and sulfur. Nitrogen and phosphorus are found both organically and inorganically, and are often present in soil, sediments, and groundwater.

Bioaugmentation is the addition of microorganisms that can biotransform or biodegrade a particular contaminant. To date, bioaugmentation has not been consistently effective in a subsurface environment. However, bioremediation can be enhanced by the continuous addition of microorganisms to a bioreactor for the above-ground treatment of contaminated groundwater. Organisms

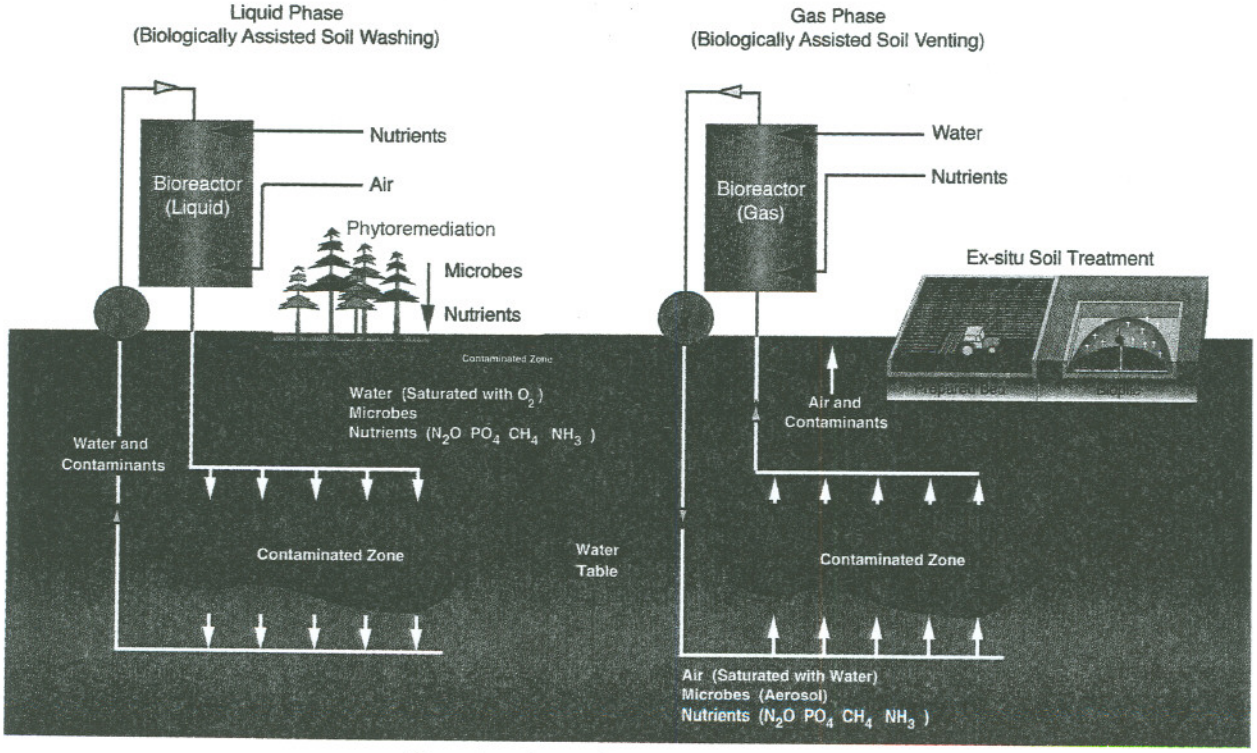

Figure 2.1. Bioremediation treatment strategies.

produced in on-site bioreactors may also be added to ex situ treatments such as engineered soil piles, or they may be injected into the subsurface for in situ treatment.

Ex situ bioaugmentation is a common technology 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 to 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. In situ bioaugmentation with GEMs is still in the preliminary testing phase in fully or partly contained systems. There is a great deal of interest in the in situ use of GEMs for the treatment of hazardous wastes. Successful agricultural field trials with GEMs (e.g., nitrogen-fixing bacteria) and genetically altered plants (e.g., herbicide-resistant soybeans) are widespread. Organisms with enhanced capabilities to degrade hydrocarbons, aromatic compounds, and halogenated compounds have already been developed.

Theoretically, organisms could also be developed to degrade or transform heretofore recalcitrant compounds such as those containing metals and radionuclides. However, the application of genetic engineering technology for use in the environment remains controversial. This is partly due to the concern that GEMs are not "natural" and may persist in the environment, potentially causing an environmental upset like the rabbit introduction to Australia. Yet, the use of GEMs may be warranted when they are the only microorganisms that can transform or degrade a particularly hazardous contaminant.

Through its Bioremediation and Its Societal Implications and Concerns (BASIC) program, DOE addresses questions and concerns surrounding the field testing and release of microorganisms for environmental cleanup. This is being accomplished through communication and collaboration with all relevant stakeholders — community leaders and representatives, engineers, scientists, and lawyers, to name a few.

Intrinsic Bioremediation

Intrinsic bioremediation occurs in situ and relies on the already-existing naturally occurring biological processes. It is also known as natural attenuation. Intrinsic bioremediation was first noticed a number of 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. In order to establish that intrinsic bioremediation is actually occurring at these rates, plume size and metabolic activity 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. Also, it is possible that scientists could take advantage of rapidly developing information on microbial processes in the subsurface, such as iron and sulfur reduction, to assess and perhaps reduce the need for the application of more costly and disruptive accelerated bioremediation technology.

Phytoremediation

Phytoremediation is the use of plants to remediate contaminated soils in the rhizosphere, which is the soil that surrounds and is influenced by plant roots and their associated microbial communities. Two forms of phytoremediation are phytoextraction and rhizofiltration. Phytoextraction is the use of metal-accumulating plants to remove toxic metals from soil. Rhizofiltration is the use of plant roots to remove toxic metals and radionuclides from contaminated waters. The plant root system serves both as a means for effective soil colonization and as a ready source of nutrients, with the result that microbial activity in the rhizosphere is greater and more easily sustained than in nonrhizosphere soil. In addition to uptake and transformation of organic compounds, many plants bioaccumulate metals and radionuclides. 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.

Phytoremediation technology has several advantages. It is inexpensive compared to conven-

tional technology and should prove cost effective for soils in which near-surface contamination is dispersed over broad areas.

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 (N-P-K) added to stimulate microbial growth. Supplemental microorganisms may also be added. Although land farming 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 land farming has been adopted to comply with revised environmental regulations.

This modified form consists of sediment 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 offsite.

Composting is a process applied to soil sediment biopiles that controls and utilizes heat generated by aerobic microbial metabolism. The material being composted serves as a source of nutrition 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, in which the sediment piles are amended 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, redox potentials, and production of biosurfactants. Any of these processes could be used to either mobilize, immobilize, or biotransform radionuclides and metals.

Slurries and Sediment Washing

Slurry bioreactors and sediment-washing equipment are commonly used to treat excavated sediment to which water is added. Slurry bioreactors are stirred tanks within which biodegradation or biotransformation takes place in an aerated environment. Sediment washing, which can be used in conjunction with the slurry process, is primarily a means of reducing the volume of contaminated sediment by solubilizing readily desorbed contaminants. It can be performed with or without accompanying biological treatment. Through rinsing, excavated sediments are screened to remove large debris, such as pipes, bricks, and concrete. Screened 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 biodegradation or biotransformation 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. Therefore, if more than one contaminant type has been released into the subsurface, multiple plumes can form with different distributions. Although a contaminated site can have a number of plumes with different contaminants or contaminant combinations, here we will examine the characteristics of a single "composite" contaminant plume (see Figure 2.2).

A source of contamination may be a single-point source such as a leaking tank. Or, the plume may have spread out from contamination of a large area, such as nitrate contamination of a water source caused by the general use of fertilizer on farm land. Point sources are frequently spills, treatment lagoons, and disposal sites such as cribs, trenches, landfills, and underground storage tanks.

Once a contaminant is released into the environment, the plume can spread into soils, unconsolidated sediments, rock formations, groundwater, and surface water. The contaminant itself may be in gaseous, liquid, or solid form, or a combination. Depending on the geology 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 groundwater or rainwater infiltration events.[1] 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 the groundwater, the shape of a plume will depend on the rate of migration, which is largely controlled by groundwater flow directions and velocity, the geologic setting, the physical and chemical characteristics of the contaminant, and the presence of a continuing source. If 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 chemical and biological reactions. These factors are briefly described below.

Advection is the transport of dissolved solutes with the bulk flow of water in the vadose zone (above the water table) or in groundwater. For highly soluble contaminants that do not undergo chemical or biological reactions with the geologic materials, advection is the primary mechanism influencing the fate and migration of the contaminant. 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. Dispersion is the mechanical mixing of solutes that occurs as the solutes are advected through the groundwater system.

1. Geohydrology 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 at DOE sites have leaked over the last 40+ 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), 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 also have an extreme influence on the heterogeneity of contaminant plumes in the vadose zone.

Biological and chemical reactions also affect the size and shape of the plume — mostly by slowing or preventing 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, that may themselves move with groundwater flow, thereby transporting the contaminant. Studies in the DOE's Subsurface Science program showed that both colloids and microorganisms accumulate at air–water interfaces.

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. Water table fluxes can thereby cause unexpected concentration phenomena. Chemical and biological interactions also can result in precipitation of the contaminant into a solid phase that is no longer mobile. Organic contaminants also can be degraded into simpler molecules. Some of these are no longer toxic, but in some cases the so-called daughter product 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.

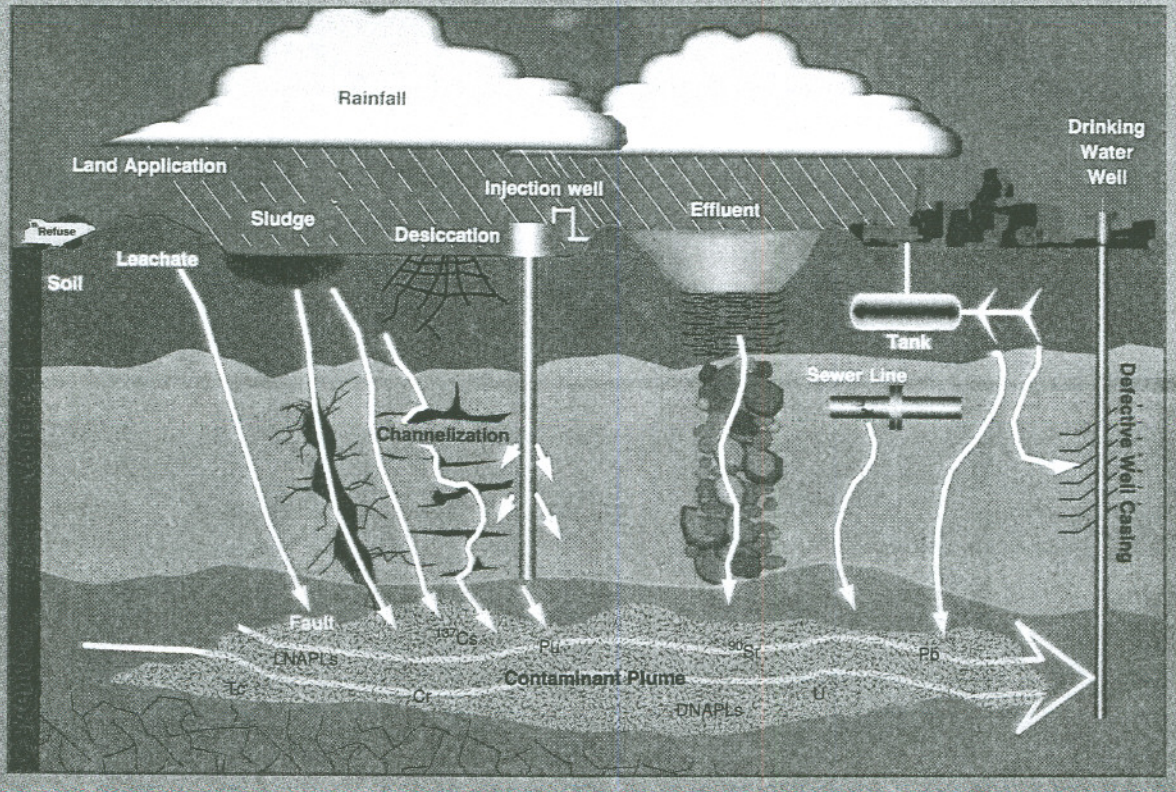

Figure 2.2. Sample contaminant plume consisting of mixed waste resulting from percolation from leaky tanks, landfills, basins, and trenches, as well as being formed through direct injection.

SECTION III:

METALS & RADIONUCLIDES FOUND AT CONTAMINATED SITES

This primer looks at ways microbial processes can be used to help remediate soils, sediments, and groundwater contaminated with metals and radionuclides. Section II provided a general introduction to bioremediation and an overview of the various bioremediation technologies. In this section, we describe several of the metals and radionuclides of most concern at many Department of Energy sites. 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 groundwater, and in soils and sediments at DOE facilities.

These metals and radionuclides are all 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 are influenced by their oxidation states,[1] solubility, and sorption processes. Transport and toxicity are both affected by contaminant form. One form or species of a metal or radionuclide may be harmless, while another can be extremely toxic. In addition, one species may be extremely mobile because it is water soluble, while another is immobile because it is in a solid phase or adsorbed to a mineral surface.

RADIONUCLIDES

Radionuclides in the environment can be present in many forms, depending on the nature of the surrounding environment. They form complexes with natural organic ligands such as humic substances. The solubility of these complexes varies with the pH of the natural aquifers. For example, compounds with hydroxides are common at high pHs.

Radionuclides also can form complexes with inorganic materials such as carbonate and sulfate. Some radionuclides are associated with colloids. At DOE sites, radionuclides such as uranium, plutonium, and strontium were found in some cases to be disposed of with organic substances such as organic acids, complexing agents (such as EDTA), and solvents, all of

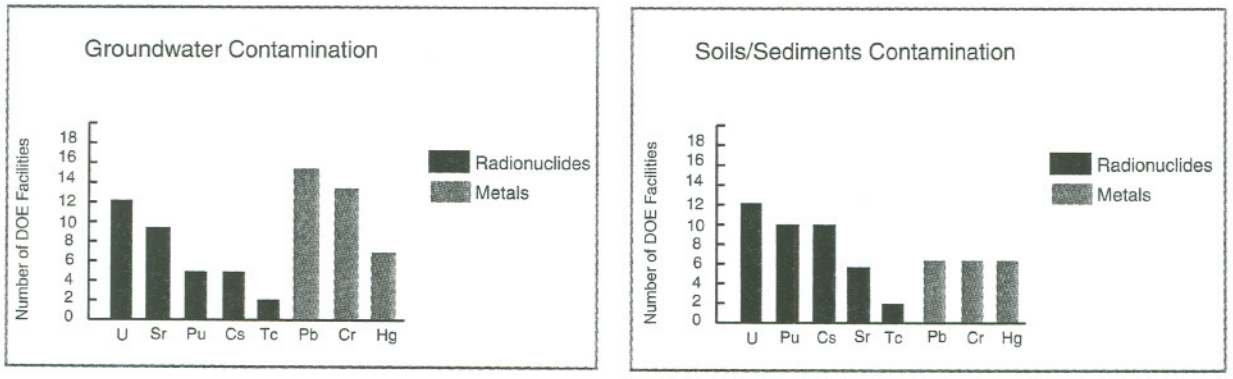

Figure 3.1. Frequency of occurrence of selected metals and radionuclides in groundwater and soils/sediments at DOE facilities (adapted from Riley et al., 1992).

1. See the feature "Opposites Attract: Valences, Bonds, and Redox Reactions" on page 23.

which can influence radionuclide geochemical behavior and subsurface transport.

Uranium and strontium have been reported in groundwater at more than 50 percent of DOE facilities, and along with tritium are the most frequent radioactive constituents in DOE groundwater. In DOE soil and sediments, uranium, plutonium, and cesium have been cited as the most common radioactive waste components (Figure 3.1).

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 weapons. 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 Ukraine. Because of cesium's similarity in chemical properties to potassium, Cs-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.

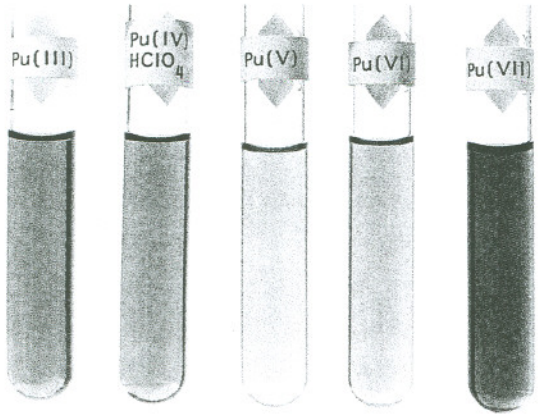

Figure 3.2. Plutonium in +3 to +7 oxidation states in colored solution, from left to right: Pu(III), Pu(IV), Pu(V), Pu(VI), and Pu(VII).

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 hydroxide (CsOH) is the strongest base known. In soils and sediments, cesium is known to sorb strongly to clays.

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 (neptunium being the first). Fifteen isotopes exist, and all are radioactive poisons because of their high rate of alpha emissions and their absorption to bone marrow. Permitted levels of exposure to plutonium are the lowest of any element.

The first isotope discovered was Pu-238 (half-life of 86 years), which was produced in 1940 by deuteron bombardment of uranium in an accelerator. The most important isotope is Pu-239, with a half-life of 24,100 years, which is produced in extensive quantities in nuclear reactors from natural uranium.

Plutonium has five oxidation states (+3, +4, +5, +6, and +7). The more insoluble form of plutonium is the Pu(IV) polymer, a hydrous plutonium oxide. However, in groundwater, the presence of complexing inorganic or organic species strongly influences 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 groundwater 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. Pu(V) is known to predominate in seawater and oxygenated lake water.

Strontium (Sr)

Strontium was first found in strontianite ($SrCO_3$), a carbonate mineral. Its other natural ore is celestite ($SrCO_4$). 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 Sr-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 the most 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.

Technetium (Tc)

Technetium was the first artificially produced element. The isotope technetium-97 (with a 2.6-million-year half-life) was discovered in 1937 in a sample of molybdenum that had been bombarded by deuterons. Technetium is a silvery-gray metal that tarnishes slowly in moist air. Nineteen Tc isotopes are now known, with atomic masses ranging from 90 to 108. All of them are radioactive.

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 groundwaters under these conditions. Less soluble sulfide, carbonate, and oxide forms of Tc(IV) represent the most dominant species under anaerobic conditions.

Technetium-99 (with a 212,000-year half-life) 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. It 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, 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 Tc-99 in these plumes is believed to be in the form of TcO_4^-. Pertechnetate can be mobile in groundwaters and biologically available, and thus constitutes a significant part of the potential radioactive dose to humans at these sites. This factor, coupled with the long half-life of Tc-99, makes the presence of this radioisotope a great concern.

Technetium-99m, not to be confused with Tc-99, has a short half-life of just over 6 hours. It is an important tracer radioisotope in nuclear medicine.

Uranium (U)

Uranium, with an atomic number of 92, is the heaviest known natural element. It is dense, hard, metallic, and silvery white. Uranium occurs in a number of minerals, including carnotite and uraninite, a dense black variety of which is called pitchblende. Uranium is not all that rare, being more plentiful in the Earth's crust than mercury and silver.

Uranium has four oxidation states: +3, +4, +5, and +6. Uranium(VI) exists in a soluble state and is very mobile in groundwater. Uranium(IV) can be stable in reducing conditions and is highly insoluble, and as particulate matter can be inhaled into the lungs. The ions in the +3 and +5 states are not as stable. Weathering of rocks converts uranium to the +6 state, where it forms the uranyl ion (UO_2^{2+}). Most uranyl compounds tend to be soluble in groundwater, although the phosphates are quite insoluble. When uranyl ions encounter a reducing agent such as organic matter, U(IV) is precipitated (separated from solution) as uraninite and coffinite.

All uranium isotopes are radioactive. 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.

Natural uranium is used in the generation of nuclear fuel, specifically in converter and breeder reactors. Uranium-235 is one of the two fissile materials used for the production of nuclear weapons, and in some nuclear reactors as a source of energy. The other is Pu-239, which is virtually nonexistent in nature and is made by bombarding U-238 with neutrons in a nuclear reactor. Because of its importance in the fission process, uranium is found in large quantities in stored nuclear waste.

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 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 soft tissue cancers.

Uranium mining to obtain yellowcake is a 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 are in the form of thorium-230 and radium-226. These waste products have a half-life of about 75,000 years and 1,600 years, respectively. They can leach into groundwater, 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.

Enrichment is another frequent source of uranium contamination. Enrichment increases the amount of uranium-235 in natural uranium for use in reactors. Uranium hexafluoride (UF_6), an interim product of the enrichment process, contains the soluble U(VI) ion and is highly radioactive and toxic. It reacts readily with moisture, releasing highly toxic hydrofluoric acid. Enrichment facilities have had a number of accidents involving UF_6.

METALS

Chromium (Cr)

Chromium in its naturally occurring form is found mainly in chromite. The mineral chromite is composed of iron, chromium, and oxygen ($FeCr_2O_4$). It is formed in the deep subsurface, which gives this mineral a stable crystalline structure and a resistance to high temperatures and pressures. Chromium is an essential trace element and has a role in glucose metabolism.[2]

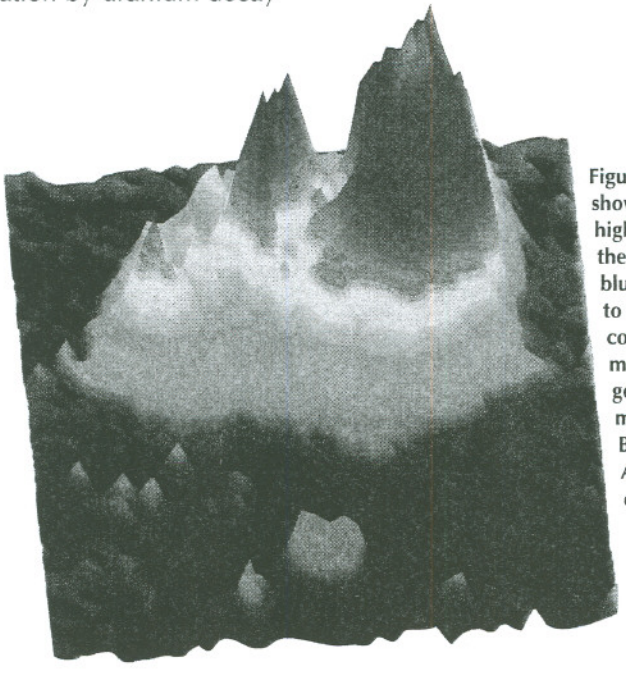

Figure 3.3. This computer image shows that chromium is present in highly localized chemical hot spots in the soil. The color scale ranges from blue, which indicates no Cr present, to red-orange, which indicates a concentration of one picogram per micrometer. The image was generated on the x-ray fluorescence microprobe beamline at Lawrence Berkeley National Laboratory's Advanced Light Source. (Courtesy of Tetsu Tokunaga.)

Chromium is found in three oxidation states (+2, +3, and +6), although in a few stable compounds it also exists in the +5, +4, and +1

2. To learn more about the chemical and physical properties of chromium and other elements, visit Britannica Online, at http://www.eb.com/ (Encyclopedia Britannica, 1998).

states. The most commonly found oxidation states are +3 and +6, with +6 being the most toxic. Trivalent chromium is an essential nutrient required in sugar and fat metabolism, and in the action of insulin. Hexavalent chromium is toxic and carcinogenic.

Chromium wastes are associated with reactor operations, fuel fabrication, and irradiated fuel processing at DOE facilities, and the toxic and soluble Cr(VI) form is reported in soils and sediments on DOE lands. Chromium(VI) can also enter the environment in effluents from metal plating and through exposure through handling and inhalation, and in industrial or municipal waste treatment plant discharges.

Chromium(VI) is highly soluble; i.e., it can dissolve easily in water and move through the subsurface environment. It has been found to cause throat and lung cancer, and long-term toxic effects may include shortened life span, reproductive problems, and lower fertility.

Chromium(VI) dissolved in groundwater is typically found in the form of CrO_4^{2-} at neutral and high pH. Under acidic conditions it occurs as $HCrO_4^-$. Dissolved Cr(III) occurs as $Cr(OH)_3$ at neutral and alkaline pH, and $Cr(OH)_2^-$ under acidic pH. Other inorganic and organic complexes with Cr(III) also occur. Solubilities of Cr(III) species are generally very low. Chromium(III) is reoxidized to Cr(VI) through redox reactions with MnO_2. Chromium precipitates occur primarily as Cr(III) compounds. Reduction of Cr(VI) to Cr(III) in sediments and groundwater can be due to organic compounds (including natural organic matter), ferrous iron [Fe(II)], and sulfides [S(–II)]. Chromium(III) sorbs strongly onto Fe and Mn oxides, clays, and other mineral surfaces. Chromium(VI) also sorbs onto Mn, and Fe oxides. Biotransformation of Cr(VI) to the less toxic and mobile Cr(III) presents an opportunity for bioremediation of chromium.

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. PbO_2 is a lead oxide that is soluble in water. Lead(II) is generally insoluble in groundwater. Lead carbonate [$Pb(CO_3)$] and lead sulfate [$Pb(SO_4)$] are insoluble 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 wastes are associated with reactor operations and lead ions are found in high concentrations in groundwater 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, children can experience behavioral changes and decreases in intelligence. At high levels, 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.

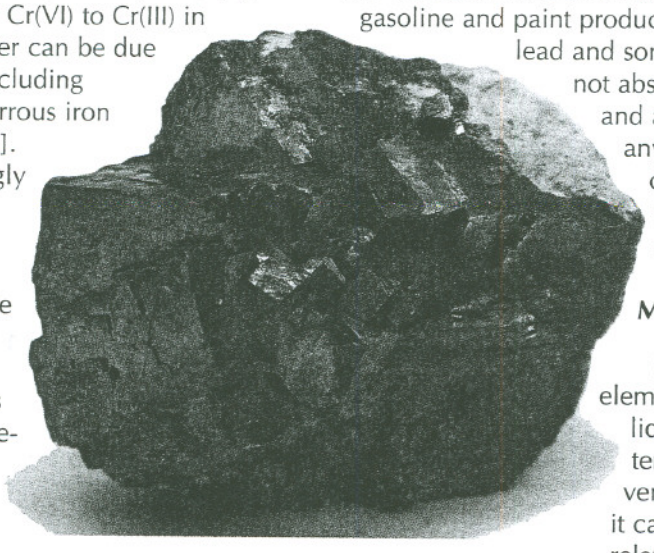

Figure 3.4. Lead is usually found with sulfur in the mineral galena (PbS).

Mercury (Hg)

Mercury is the only elemental metal that is liquid at room temperature. Mercury is very volatile, meaning that it can readily vaporize at relatively low temperatures. In its solid form, it is silvery white, slowly tarnishing in

moist air, and freezing into a soft solid like tin at about -39°C (-38.2°F). It alloys with most metals. Mercury's principal ore is the red sulfide, cinnabar (HgS).

Inorganic mercury exists in three oxidation states: 0 (elemental mercury); +1 (mercurous mercury); and +2 (mercuric mercury). Mercury compounds contain either the Hg(I) or Hg(II) ion, although Hg(II) compounds predominate. All three oxidation states 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 readily 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.

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

The major form of mercury in the atmosphere is elemental mercury, which is highly volatile. Burning of fossil fuels contributes to this atmospheric contamination. Even though elemental mercury, Hg(0), is the least reactive of the three oxidation states, it 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.

The mercuric ion readily adsorbs to these sediments and other particulate matter. Anaerobic sulfate-reducing bacteria, which commonly inhabit sediment, can methylate this 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.

Although methylmercury is highly toxic, bacteria have evolved genes that convert it to a much less toxic form. Thus, methylmercury is a suitable candidate for bioremediation. 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. 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.

OPPOSITES ATTRACT:
VALENCES, BONDS, AND REDOX REACTIONS[1]

Atoms bond to achieve stability. Chemical bonds are formed through the giving up, receiving, or sharing of electrons by the outermost region of an atom, called its valence shell. The valence-shell electrons are the least tightly bound to the nucleus and therefore can be removed the most easily.

The oxidation number of an atom is the net charge on an atomic species. And the atom's oxidation state (also known as its valence) is the number of electrons an atom can give up or receive to achieve a bond. The oxidation state of any atom is indicated by a roman numeral following the name of the element. Thus, iron(III), or Fe(III), means iron in an oxidation state of +3. The uncombined Fe(III) ion is 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 occurs from one atom to another. This creates two ions with opposing electric charge. The transfer 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 will receive the electron or electrons and become a negative ion, an anion. Most metals easily combine with oxygen to form metal oxides, and many ores consist of metal oxides. Oxygen in its ionic state has a valence of –2. Electrostatic attraction between the ions of opposite charge holds them together, creating a compound.

When atoms of two elements of about the same electronegativity react, they form bonds in which the electrons are shared about equally between atoms. Bonds formed by the sharing of electrons are covalent bonds. Covalent bonds between identical atoms (such as H_2) are nonpolar, or electrically uniform, whereas those between unlike atoms are polar, that is, one atom is slightly negatively charged and the other is slightly positively charged. This partial ionic character of covalent bonds increases with the difference in the electronegativities of the two atoms.

Oxidation–reduction, or "redox," is a chemical reaction in which there is either a complete transfer of electrons (creating an ionic bond), or a sharing of electrons with other atoms (creating a covalent bond). This changes the oxidation state of the atoms involved in the reaction. The atom that loses an electron is oxidized, and the atom that gains an electron is reduced. 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.

1. To learn more about this topic, see *General Chemistry: Principles and Structure* (Brady, 1990).

SECTION IV:

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 with diverse sources of carbon and energy. Microorganisms are so named because they are usually too small to see with the naked eye (which is about a tenth of a millimeter).

In a typical gram of sediment, 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 stems, leaves, and roots of plants — leading to the endless 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 it is hot, salty, and food is in short supply.

Microorganisms span the three domains of life: Bacteria, Eukarya, and the recently recognized Archaea. These three domains are divided according to the structure of their cells. The cells of higher animals and plants are eukaryotic and have a true nucleus. The ancestors of multicellular organisms are eukaryotic microorganisms. 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.[1]

Microorganisms also can 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. In sediment and groundwater 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 transformations 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 that 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 others, it is transformed while a second substance serves as a primary energy or carbon source. This cometabolism process may be purely fortuitous, and the microorganism gains nothing from the process. Contaminant degradation may result in daughter products that can be metabolized or in ones that persist.

Transformation of metals and radionuclides proceeds somewhat differently. Although they cannot be sources of carbon, metals and radionuclides can provide energy, and they can also

1. For more information about how scientists identify microorganisms, see the feature "Who's Out There? Identifying Microbial Species that Live in the Subsurface" on page 29.

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 co-factors to enzymes. They can also be transformed indirectly by reducing and oxidizing agents produced by the microorganism that cause changes in pH or redox potential.

Transformation may also occur when microorganisms produce chelating agents that bind the metal or radionuclide or degrade the chelating 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 biotransformation of metals and radionuclides.[2]

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 constructive (anabolic) processes. Catabolic processes break down larger molecules into simpler components, producing energy for microbial growth and reproduction. Contaminants can be transformed into less harmful forms or degraded completely (mineralized) to inorganic components through these catabolic processes. Some of the most important components of catabolism are nutrient and energy sources; microbial respiration; basic respiratory oxidation–reduction reactions, which generate energy and transfer electrons from electron donors to electron acceptors; and enzymes, which serve as catalysts to these reactions.

Nutrient Sources

Carbon, nitrogen, and phosphorus are the major nutrients needed by the cell. This is because they are the basic elemental components of the proteins, sugars, and nucleic acids that comprise the cell. 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 gas as a nitrogen source, fixing it directly from the air.

Microorganisms also need other nutrients, although to a lesser extent. Production of ATP (adenosine triphosphate — the principal energy carrier molecule of the cell) and synthesis of nucleic acids and phospholipids require phosphorus, which occurs in nature in the form or organic and inorganic phosphates (PO_4^{3-}). 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

Microorganisms can use two sources of energy other than organic compounds — light and inorganic chemicals. Those that use light are phototrophs, converting that light energy to

2. To learn more about microbial metabolism, see *Brock Microbiology of Microorganisms* (Madigan et al., 1997).

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 metals and radionuclides as primary sources of energy are chemolithotrophs, that is, they use inorganic chemical compounds as an energy source.

Microbial Enzymes Acting as Catalysts

Enzymes are proteins that catalyze chemical reactions in the cell. One of the most important of these reactions is oxidation–reduction (redox) in catabolic metabolism. These redox reactions transfer electrons and release energy from a substance. The substance that an enzyme acts upon is called the reactant, or substrate. This is often the contaminant in bioremediation. A specific enzyme-catalyzed reaction is usually only one of many of the reactions in a catabolic or anabolic pathway.

For a reaction to even occur, molecules must first reach a reactive state in order 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 this by lowering the amount of activation energy needed to initiate a reaction. They 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 what would occur spontaneously.

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:

$$E + S \leftrightarrow E\,S \leftrightarrow E + P$$

The combination of enzyme and substrate usually depends on weak bonds to join the enzyme to the substrate. To catalyze a reaction, an enzyme must bind the correct substrate and position it correctly on 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 activation energy required to make the reaction occur and transform the substrate. Enzymes are 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 decomposes 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.[3] This transfer occurs through the donation of one or more electrons from an energy source (substrate), called the electron donor, and accepted by the electron acceptor, leading 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 subsequent 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

3. See the feature "Opposites Attract: Valences, Bonds, and Redox Reactions" in Section III.

Table 4.1.
Microbially Significant Half-Reaction Reduction Potentials

Redox Pairs	E_0(V)
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	+1.229*
$MnO_2(s) + 4H^+ + 2e^- \rightarrow Mn^{2}+ + 2H_2O$	+1.208*
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	+0.94†
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	+0.77*
$SO_4^{2-} + 4H^+ + 2e^- \rightarrow H_2SO_3 + H_2O$	+0.20*
$2H^+ + 2e^- \rightarrow H_2$	0.0†

*Oxtoby et al., 1994; † Tinoco et al., 1985.

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 (E_0). 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 bioremediation of metals and radionuclides.

In soil and groundwater systems with abundant carbon and nutrients for microbial activity, there is a well-defined sequence of redox reactions that occurs (Sposito, 1989). 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, dissolution of Fe^{3+} minerals to aqueous Fe^{2+} occurs 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. Fortunately, as described in Sections V and VI, the reaction products of these major earth elements can also react with some radioactive and metal contaminants to form stable mineral phases.

Microbial Respiration

Respiration is a fundamental metabolic process whereby microorganisms obtain the energy needed to grow and reproduce. There are two basic divisions of respiration: aerobic and anaerobic. Aerobic respiration occurs when the terminal electron acceptor is O_2. Anaerobic respiration is the use of inorganic compounds other than O_2 as terminal electron acceptors.

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 culturing, microbiologists have been able to grow, at most, 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 ways of identifying microbes and assessing the microbial communities in the subsurface.

A community can be assessed directly by isolating DNA and after amplification (see below), determining the sequences of specific genes. After identification, the sequence can be compared to a large database comprising 16S rRNA sequences of previously cultured organisms. The patterns obtained from the fatty acid methyl esters (FAME) of organisms grown under carefully controlled conditions can be used for culture identification. Analogous to the FAME analysis, by carefully identifying specific lipid molecular classes[1] and focusing on the fatty acids of polar phosphate-containing lipids, the community can be further characterized. The total microbial community can be examined, but no one method can furnish a complete analysis. Along with culturing, however, each of these approaches provides a piece of the puzzle.

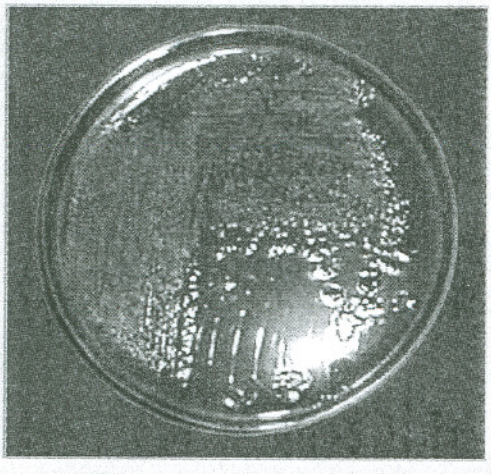

Figure 4.1. Culture of *Pseudomonas stutzeri* on a plate. Image courtesy of F. Blaine Metting, Pacific Northwest National Laboratory.

Culturing 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. Sugars and amino acids may be added to the medium, as well as some kind of solidifying agent, like 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 FAME patterns and RNA sequences can be used for further confirmation.

16S rRNA Gene Sequencing. This identification method can be used with archaea as well as eukarya and bacteria. It is based on determining the phylogenetic position of the unknown microbe among known microorganisms. This determination is based upon a particular DNA strand — its 16S rRNA gene sequence. This sequence is considered the best for these evolutionary measurements because it is highly conserved.

1. 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.

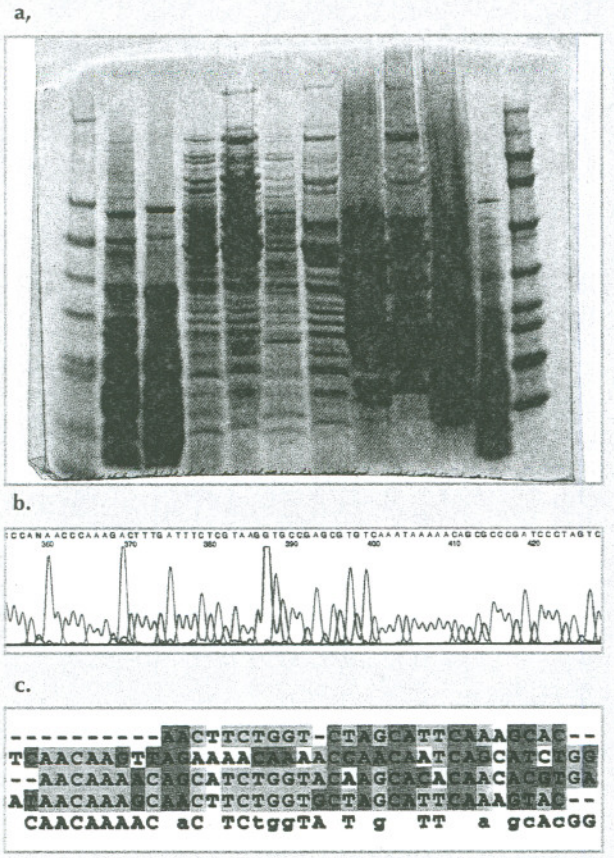

Figure 4.2. (a) RNA sequences are separated by gel electrophoresis. (b) Sequencing results are color coded by base type (adenine — green, guanine — black, cytosine — blue, and thymidine — red). (c) Alignment of four sequences, color coded to denote matching bases. Images courtesy of Tamas Torok, Center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

Obtaining the 16S rRNA sequence is accomplished in a variety of ways. One of the most common and effective is PCR (polymerase chain reaction), [2] which replicates the 16S rRNA strand. This amplified material is then sequenced. Next, the sequenced 16S rRNA is compared to the sequences of other microorganisms that have been placed in a database created by Carl R. Woese and Gary Olsen at the University of Illinois (the Ribosome Database). Drs. Woese and Olsen have structured all three classes of organisms into relationships with one another based on the differences between the nucleotides in their 16S rRNA strand (Figure 4.2). Pairs of sequences from different organisms are aligned, and the differences in their nucleotide sequences are counted. The number of differences form a basis for measuring the evolutionary distance between organisms. (See the inside back cover for a phylogenetic tree based on the Ribosome Database.) In addition, knowing the phylogenetic position of an unknown, uncultured organism can sometimes allow inference of its physiological properties, which in turn can suggest culture conditions that allow its isolation.

FAME (Fatty Acid Methylester) Analysis. This approach is used to identify unknown bacteria through characterization of the fatty acid composition of the lipids in the cell membrane. For the FAME analysis, bacterial cell material is hydrolyzed, and then saponified in sodium hydroxide. The material is then acidified with hydrogen chloride in methanol, causing the fatty acids to be methylated to form methyl esters. The fatty-acid-methylated esters are then extracted with an organic solvent, and injected into a gas chromatograph. After obtaining the gas chromatogram profile of an isolate, with peak identification by mass spectrometry (Figure 4.3), its FAME profile can be compared to those of known organisms in the 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.

Signature Lipid Analysis. This approach is based on extraction of the lipid components of the 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.

2. A new technology that can now enzymatically amplify minute quantities of specific gene fragments millions of times.

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. The purple staining gram-positive organisms have a PLFA pattern much different than the red staining gram-negative bacteria. Certain groups such as the actinomycetes, the Archea, and the sulfate-reducing bacteria can be identified by their distinct patterns. Higher microbes, such as algae, protozoa, and fungi can also be identified. 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., physiological/nutritional status can be determined. Consequently, PLFA analysis provides the viable biomass, composition, and nutritional/physiological status of the community. All of these allow investigators to ask not only who is out there but what the conditions are at the site where bioremediation is to be done. In using PLFA analysis we are "asking the microbes" if the various manipulations are effective. We can then utilize shifts in their ecology as a comprehensive and integrated monitor for toxicity assessment.

Recently, the signature lipid analysis has been expanded in research supported by NABIR 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 augmentation by bacteria to enhance bioremediation.

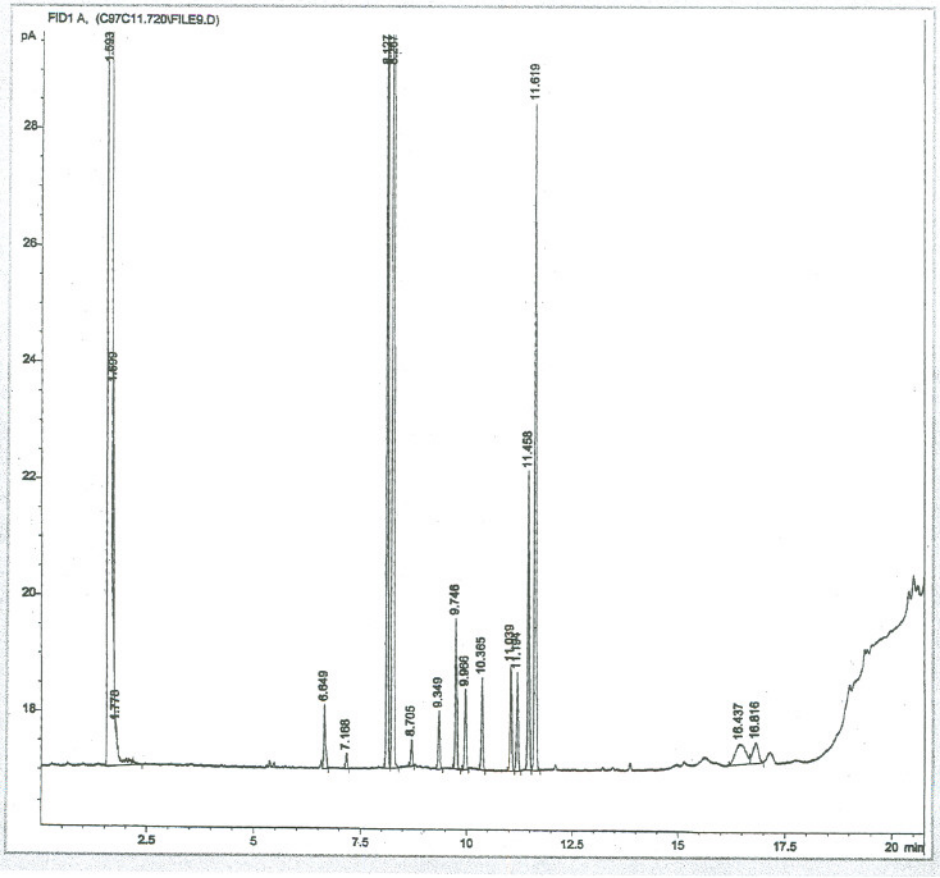

Figure 4.3. FAME chromatogram showing chromatographic column retention times and peak heights of a microorganism isolated from subsurface rock cores at Idaho National Engineering and Environmental Laboratory INEEL-10 test site. The 1.593 (far left) peak is the solvent peak. Remaining are carbon fatty 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, Lawrence Berkeley National Laboratory.

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 the synthesis of more 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 as 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. This will make the contaminant less likely to enter groundwater.

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.[4] 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 these electron acceptors have as large a reduction potential as the O_2/H_2O couple (Table 4.1), less energy is released when they are used.

When inorganic compounds such as nitrate (NO_3^-), sulfate (SO_4^{2-}), and carbon dioxide (CO_2) are reduced for use as nutrient sources, they are said to be assimilated, and the reduction process is called assimilative metabolism. When they 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 the nutritional needs, and the reduced atoms are converted to cell material. In dissimilative metabolism, a relatively large amount of the electron acceptor is reduced, and the reduced product is excreted 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 food 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 is catalyzed by nitrite reductase and follows one of two paths: either through nitric oxide (NO) or nitrous oxide (N_2O).

If oxygen is removed from a system and nitrate is present, denitrification will occur to the exclusion of most other metabolisms. Denitrification provides microbes with a relatively high amount of energy, and microbial growth rates are consequently high compared to other anaerobic metabolisms.

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 be important to biological treatment of metals and radionuclides by inhibiting the activity of dissimilatory iron reduction or sulfate reduction, causing an increase in pH or depleting substrate. Denitrifiers can be integral to an in situ biological treatment approach if nitrate is one of the contaminants.

Most denitrifiers are facultative aerobes, that is, they can switch to denitrification when O_2 is no longer available as an electron acceptor. The bacteria *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, and *Pseudomonas aeruginosa* are three such denitrifiers.

4. Many of the microorganisms involved in anaerobic respiration are extremophiles — they can exist at extremely hot temperatures, in salty bodies of water, and in environments with extreme variations in pH. For more information, see the feature "Extremophiles: Microscopic Exotica" on page 34.

Iron reduction. The reduction potential of Fe^{3+}/Fe^{2+} is very electropositive (Table 4.1). Several microorganisms are able to couple oxidation of hydrogen or organic compounds to the reduction of Fe^{3+} and gain energy for growth. These bacteria include species from several genera, including *Geobacter*, *Desulfuromonas*, *Pelobacter*, *Shewanella*, *Ferrimonas*, *Geovibrio*, *Geothrix*, and *Bacillus*. These organisms have a broad spectrum of other metabolic capabilities as well. For instance, *Shewanella* species can use oxygen, nitrate, uranium, manganese, and iron as electron acceptors.

The use of Fe^{3+} and other metals by certain microbial groups as terminal electron acceptors for anaerobic respiration is of particular relevance to bioremediation of heavy metals and radionuclides. Dissimilatory iron reducers and other microorganisms can reduce mineral-associated iron to produce reactive sites within the minerals or to directly reduce contaminants, such as uranium and chromium. A number of species are able to reduce structural iron, even in amorphous minerals such as ferrihydrite, and crystalline iron oxy-hydroxides, including the minerals hematite, goethite, and magnetite.

Sulfate reduction. Sulfate (SO_4^{2-}) is the most common sulfur compound 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 are lower. The first product of sulfate reduction is sulfite (SO_3^{2-}). The end product is hydrogen sulfide (H_2S). Usually, organic carbon compounds are the primary electron donors in sulfate reduction. But 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. However, most sulfate-reducing organisms are chemoorganotrophs, using various organic compounds as electron donors, including the fermentation (see below) products lactate, acetate, and ethanol.

The metabolic activity of sulfate reducers is not limited to the reduction of sulfate; other metals can be reduced by 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. The microorganisms in cows are prolific methane producers. 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.

Extremophiles: Microscopic Exotica

Life can be found almost everywhere on this planet. Much of this life exists in the form of microbes. And the most exotic microorganisms, extremophiles, can live in niches where no other organisms are found. Thermophiles characteristically grow at temperatures greater than 45°C (113°F). Hyperthermophiles can live in environments with temperatures of 80°C or higher. Some extremophiles can tolerate pH levels less than two or greater than ten. And halophiles exist in saturated saline. A number of these microbes belong to the newly defined domain of life, the Archaea. Scientists first believed extremophiles were predominantly archaea, but now they are starting to see bacteria in these extreme environments as well. For example, samples taken from Yellowstone Park's Obsidian Pool showed the ratio of thermophilic bacteria to archaea as 50 to 1 (Pace, 1997). This hot-temperature environment is high in hydrogen sulfide, iron, hydrogen, and carbon dioxide.

Extremophiles can be a boon to bioremediation. Many extreme environments are anaerobic, so these microbes do not need oxygen. They can survive environments that are similar to toxic waste sites and would poison or kill other organisms. Proteins from some of these extremophiles are presently being isolated and characterized in the hopes of learning how they function in such extreme environments. Hopefully, this information will be helpful in re-engineering other microorganisms so that they can tolerate extreme conditions.

Below are profiles of several interesting extremophiles.

Methanococcus jannaschii was the first archaeon whose genome was sequenced (Bult et al., 1996). It was first isolated at the base of a Pacific thermal vent off the coast of Baja California in 1983. *M. jannaschii* possesses a small (about 1.66 Mbp) genome. It is a methanogen (methane producer) and a thermophile. This microbe normally lives at about 8,000 feet below sea level, where the pressure equals about 230 atmospheres (3,380 pounds per square inch). It is strictly anaerobic and autotrophic.

Bacillus infernus (the "bacillus from hell") is a newly identified species of bacteria (Boone et al., 1995). This is the first-known strictly anaerobic member of the bacterial genus *Bacillus*, which prior to this had always been described as aerobic. This thermophile was obtained from a depth of about 9,000 feet below the land surface. Microbes at this depth have been in isolation from the surface for millions of years and have evolved very exotic metabolisms and slow rates of reproduction.

Deinococcus radiodurans species can withstand exposure to radiation levels up to 1.5 million rads (500 rads is lethal to humans). At that point its chromosomes shatter into hundreds of fragments. It is believed that *D. radiodurans* has a more active DNA-repair mechanism than other microorganisms because the conditions under which it is able to survive are so damaging to other species. It isn't exactly clear how *D. radiodurans* obtained its remarkable radiation resistance. It was first observed in the 1950s in cans of meat that had been exposed to supposedly sterilizing doses of radiation. The microbe has certain possibilities for bioremediation. Conceivably, a strain of *D. radiodurans* modified with genes from other organisms having bioremediation ability could be used to treat highly radioactive waste.

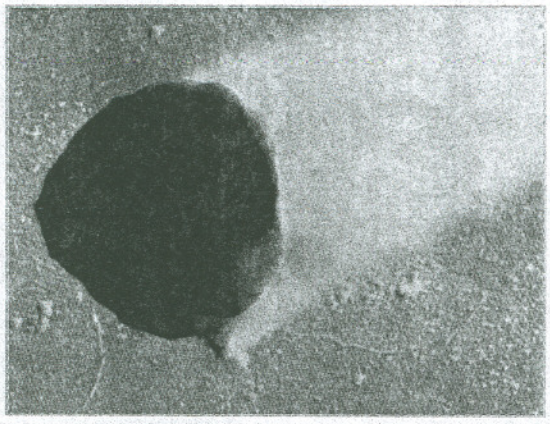

Figure 4.4. *Methanococcus* species. K. O. Stetter, Universität Regensburg, Faculty of Natural Sciences.

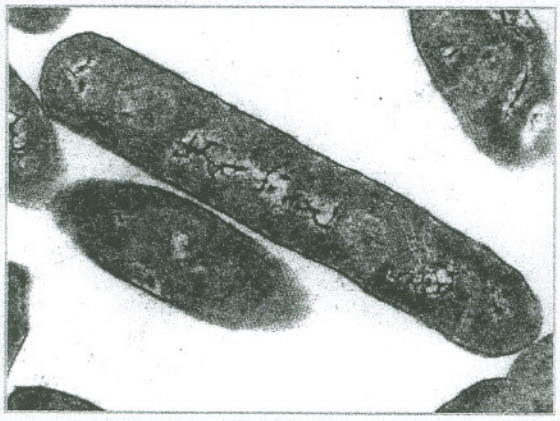

Figure 4.5. New species *Bacillus infernus*, "the bacillus from hell," magnified 50,000 times. Transmission electron micrograph taken by Henry C. Aldrich, University of Florida.

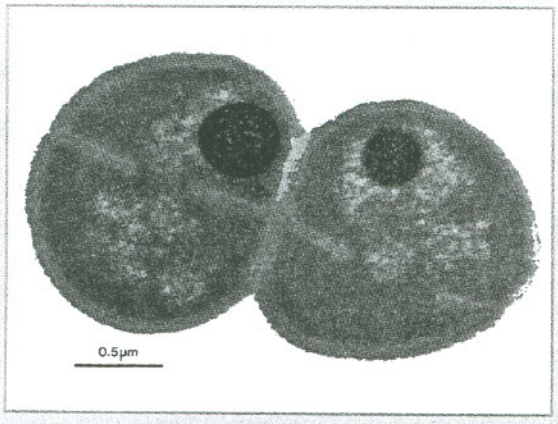

Figure 4.6. *Deinococcus radiodurans*, magnified 60,000 times. Taken by John Battista and Peggy O'Cain of Louisiana State University.

Microbial Consortia

Microbial biotransformation and biodegradation can occur only if microorganisms are present that can metabolize the contaminant. In particular, there must be microbial enzymes that can act as catalysts for the oxidation–reduction reactions that will degrade or transform the compound. However, knowing and capitalizing on the relationship of the organisms to the substrate is only one aspect of bioremediation. Another aspect is understanding the interrelationships of the microorganisms in the microbial community, or consortium. A consortium is a relatively stable but loose-knit association of microorganisms in an environment. Microbe-to-microbe interactions are complex, and may run the gamut from symbiosis to predator–prey relationships.

One type of microbial consortium is a biofilm (Figure 4.7). Biofilms are created by groups of microorganisms adhering to a sediment particle or other surface and releasing exopolysaccharides. In fact, this phenomenon may create small "stagnant" areas in the sediment pore spaces where all of the oxygen is depleted, even though the fast groundwater flow path areas and therefore the bulk environment are saturated with oxygen or are aerobic. These biofilms also allow organisms to come into juxtaposition so that a variety of complex relationships, as discussed below, can develop.

In symbiosis, two species form an association whereby the individuals of one or both species are benefited. Two of the most common symbiotic relationships are commensalism and mutualism. Commensalism is a symbiotic relationship in which a one-sided association is formed between two species. The individuals of one provide sustenance to those of the other. Neither group, however, is harmed. In mutualism, both species benefit from each other's products.

Syntrophy ("mutual feeding") is a well-known form of mutualism in which members of two species are nutritionally dependent on one another. In a syntrophic relationship, the organisms together can degrade a substance that neither can degrade separately. For example, in the coupled reaction of ethanol fermentation with methanogenesis, a syntrophic relationship is formed between an ethanol fermenter and a methanogen. The ethanol fermenter produces hydrogen (H_2) and acetate, but the energy yield from that reaction is low. The methanogen then consumes the H_2 from the fermentation half reaction to produce methane with a relatively high energy yield. The coupled reaction produces a higher energy yield for the fermentation half reaction. Therefore, the ethanol fermenter

utilizes more of the ethanol in the coupled reaction than it would without the syntrophic relationship with the methanogen. And the methanogen gets the H_2 it needs to produce the methane.

In predator–prey relationships, the first microbe consumes a substrate, and then the second microbe consumes the first microbe. The interactions of bacteria and protozoa (unicellular eukaroytic microorganisms) are an example of such a relationship. Protozoa that consume bacteria and excrete material that is readily utilizable by the same or other bacteria in the biofilm can have a dramatic effect on the rate and type of biodegradation/bioremediation. High rates of protozoa predation at sites being bioremediated by injection of bacteria could decrease the effectiveness of the treatment. However, other sites may benefit from high rates of predation by increasing turnover rate and thereby the biodegradation rate of the contaminant. High rates of predation may also lower the overall numbers of bacteria, even though the activity has increased. This gives the false impression that bacterial densities have decreased and therefore the bioremediation of that subsurface environment has declined. Other relationships between two species can also have both positive and negative effects on bioremediation.

In most situations, the microbes capable of metabolizing the contaminant are already present in the targeted area. However, if the contamination is recent or if the contaminants are complex, anthropogenic compounds (xenobiotics), or compound mixtures, there is a greater chance that capable microorganisms will not be present. And even if the right microbes are present, there may not be enough for a successful cleanup. This can be due to environmental conditions unsuitable for microbial proliferation and activity within the desired time frame to comply with government regulations.

If that is the case, commercially available microbial inoculants can be added through bioaugmentation (discussed in Section II). Inoculants usually consist of a sample of this microbial community that is extracted and cultivated in the laboratory. Conditions are then manipulated ex situ to encourage the growth of the suitable microbes, and then the conditions are duplicated in the field. In this way, organisms that are dormant or are in insufficient quantities but are specifically suited for the bioremediation of a particular contaminant can be selected. One of the major challenges to bioaugmentation is survival of the introduced microorganisms in the contaminated environment. Native or indigenous microbes may out-compete the introduced organisms for limited nutrients.

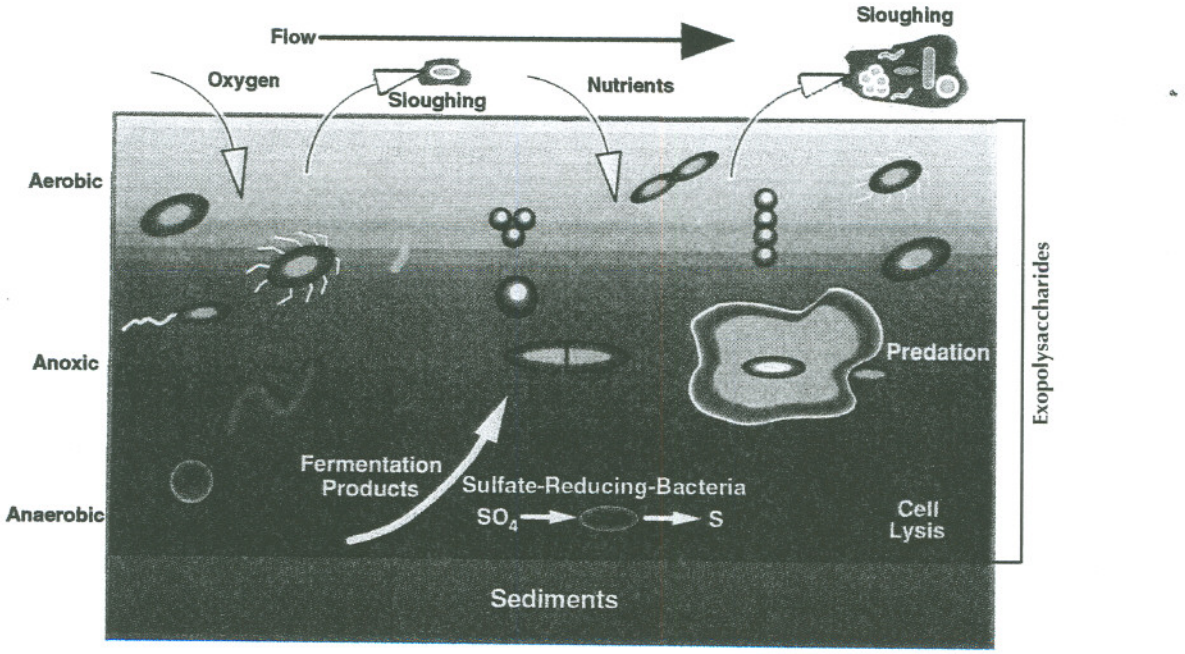

Figure 4.7. Mature biofilm.

MICROBIAL PROCESSES

AFFECTING THE BIOREMEDIATION OF METALS AND RADIONUCLIDES

In Sections II through IV we described the basic ingredients for the bioremediation of metals and radionuclides — microbial metabolism, chemical speciation and valence status, and transport processes. Here we describe how scientists and engineers believe these ingredients can be combined to bioremediate contaminated sediments and groundwater. However, because bioremediation of metals and radionuclides relies on a complex interplay of these processes and our understanding of them is just developing, our descriptions are often more qualitative than quantitative. Over the next decade we expect to gain a fundamental, mechanistic understanding of the coupling between microbial metabolism, chemical reaction, and transport — and how these work together to bioremediate metals and radionuclides. However, for now researchers are building on processes that can be understood and observed with the tools currently available.

Bioremediation of metals and radionuclides is achieved through biotransformation. Like biodegradation of organic components, it involves the breaking and creating of chemical bonds that alter the molecular species of the contaminant. This leads to changes in the solubility, sorption characteristics, transport properties, and toxicity of the metal or radionuclide. There are at least three categories of microbial processes that can influence the toxicity and transport of metals and radionuclides: biosorption and bioaccumulation; biologically catalyzed redox reactions that lead to immobilization; and biologically catalyzed solubilization. Each of these provides the potential for either mobilizing or immobilizing metallic and radioactive contaminants in the environment.

BIOACCUMULATION AND BIOSORPTION

Microorganisms can physically remove heavy metals and radionuclides from solution through either bioaccumulation or biosorption. Bioaccumulation is the retention and concentration of a substance by an organism. In bioaccumulation, metals are transported from the outside of the microbial cell, through the cellular membrane, and into the cell cytoplasm, where the metal is sequestered and therefore immobile (Figure 5.1.a).

Biosorption does not consume cellular energy. Positively charged metal ions are sequestered — primarily through the adsorption of metals to the negative ionic groups on cell surfaces, the polysaccharide coating found on most forms of bacteria, or other extracellular structures such as capsules or slime layers (Figure 5.1.b). Binding sites on

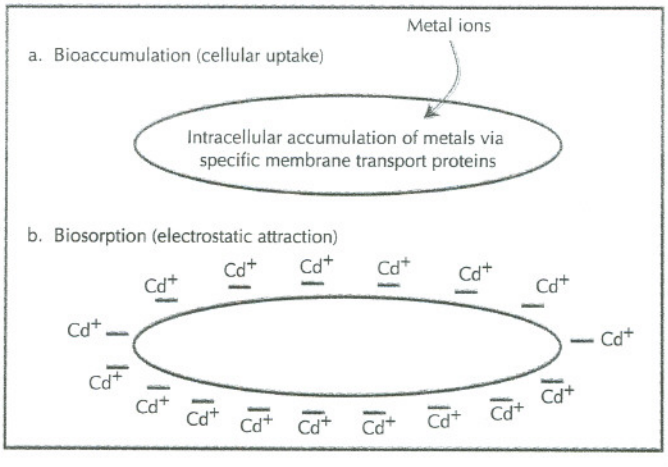

Figure 5.1. Accumulation of heavy metals and radionuclides by bacteria. (a) Metabolically active cells that express metal transport proteins can sequester metal ions intracellularly. (b) Negatively charged bacterial surfaces electrostatically attract metal cations.

microbial cell surfaces usually are carboxyl residues, phosphate residues, S–H groups, or hydroxyl groups. 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.

Biologically Catalyzed Redox Reactions that Lead to Immobilization

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), chromium (Cr), and technetium (Tc). Direct enzymatic reduction involves use of the oxidized forms of these contaminants as alternate electron acceptors. The oxidized forms of these three metals are highly soluble in aqueous media and are generally the most mobile species in aerobic groundwater, while the reduced species are highly insoluble and precipitate from solution. Direct enzymatic reduction of soluble U(VI), Cr(VI), and Tc(VII) to insoluble species has been documented and is illustrated in Figure 5.2. Extracellular precipitation of enzymatically reduced Tc by *Shewanella putrefaciens* is illustrated in Figure 5.3. Studies have also found that bioreduction of hexavalent chromium can occur through aerobic and anaerobic conditions. A number of Cr(VI)-reducing microbial strains have recently been isolated from chromate-contaminated waters, soils, and sediments, including *Oscillatoria* sp., *Arthrobacter* sp., *Agrobacter* sp., *Pseudomonas aeruginosa* S128, *Chlamydomonas* sp. (algae), *Chlorella vulgaris* (algae), *Zoogloea ramigera*, and anaerobic sulfate-reducing bacteria.

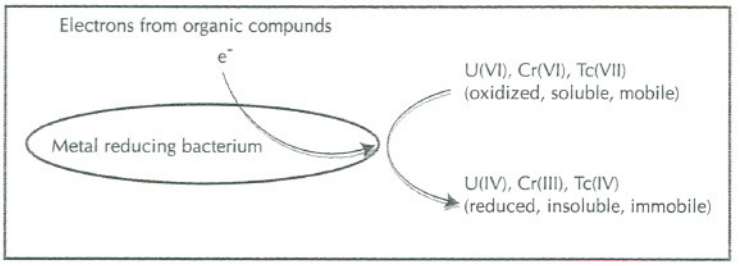

Figure 5.2. Direct enzymatic reduction of soluble heavy meals and radionuclides by metal-reducing bacteria. Nonhazardous organic compounds, such as lactate or acetate, provide electrons used by these microorganisms. Note, however, that if complexed the reduced species may become mobile.

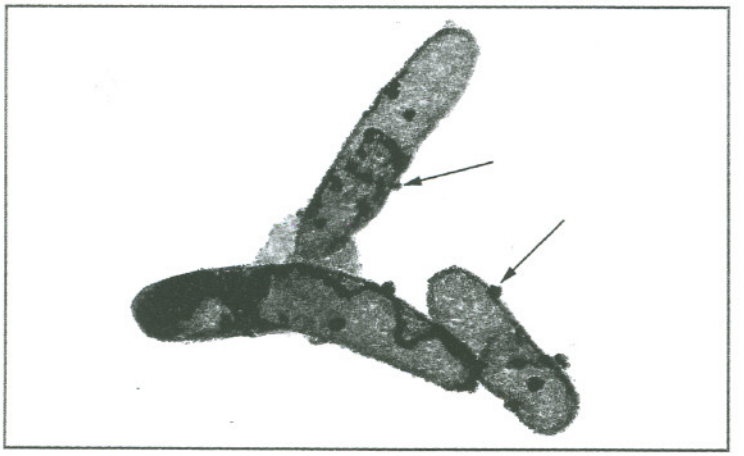

Figure 5.3 This image demonstrates the ability of *Shewanella putrefaciens*, a bacterium isolated from the deep subsurface, to enzymatically reduce and precipitate technetium (dark regions on the cell surface indicated by arrows). This phenomenon offers potential for in situ treatment of radionuclides at DOE sites. Image taken by J. A. McKinley and A. E. Plymale as part of a project led by R. E. Wildung and Y. Gorby at Pacific Northwest National Laboratories.

Metal-reducing organisms reduce uranyl carbonate, which is exceedingly soluble in carbonate-bearing groundwater, to highly insoluble U(IV), which precipitates from solution as the uranium oxide mineral uraninite. Recently, scientists have had success in microbial binding of U(VI), which is then converted by the living cells to U(IV) and precipitated intracellularly. A wide range of bacteria, including *Enterobacter cloacea* and all known metal-reducing bacteria, reduce the highly soluble chromate ion to Cr(III), which under appropriate conditions precipitates as $Cr(OH)_3$. Metal-reducing bacteria also reduce oxidized technetium, Tc(VII) (which

can be found in soluble sodium pertechnetate) to Tc(IV), forming the Tc oxide mineral TcO_2.

Although some microorganisms can enzymatically reduce heavy metals and radionuclides directly, indirect reduction of soluble contaminants may be more feasible in natural sedimentary and subsurface environments. This indirect immobilization could be accomplished by metal-reducing and sulfate-reducing bacteria. This can be achieved by coupling the oxidation of organic compounds or hydrogen to the reduction of ferric iron [Fe(III)], Mn(IV), or sulfate (SO_4^{2-}). Iron(III) is reduced to iron(II), manganese(IV) to manganese (II), and SO_4^{2-} to hydrogen sulfide (H_2S). The reduced form then chemically interacts with the contaminants and forms separate or multicomponent insoluble species.

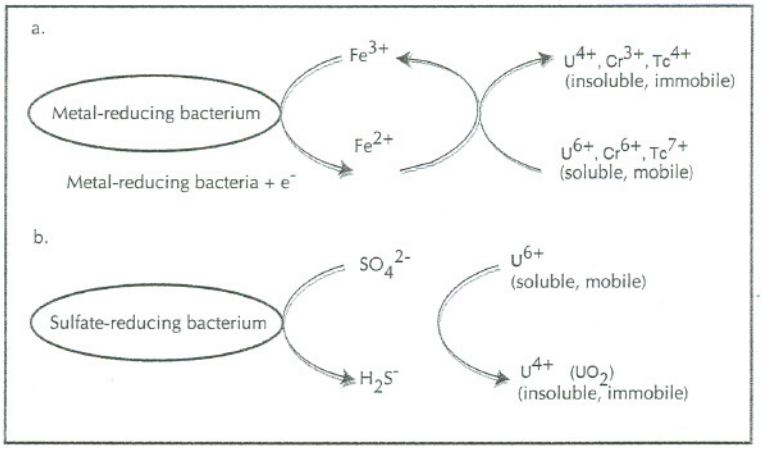

Figure 5.4. Indirect mobilization of heavy metals and radionuclides by (a) metal-reducing and (b) sulfate-reducing bacteria.

The most reactive of these reduced forms 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 5.4.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 become incorporated in metal oxide minerals as they precipitate from solution. Although the use of Fe(II) as an electron donor for reduction and precipitation of multivalent metal contaminants has been examined in the laboratory, field tests evaluating its potential as a remediation technology have not been conducted.

Sulfate-reducing bacteria also may be stimulated to produce a chemically reactive redox barrier (Figure 5.4.b). Hydrogen sulfide generated by sulfate-reducing bacteria could chemically reduce the contaminant directly, or indirectly in the case of sulfide minerals such as pyrite that would be chemically stable for extended periods of time.

Synthetic chelators such as EDTA and NTA can form stable, soluble complexes with heavy metals and were commonly used as cleaning agents during industrial processing of nuclear fuels throughout the DOE complex. Metal–chelate complexes have entered the environment and may migrate freely in groundwater. When conditions necessitate immobilization of the contaminant, one approach for limiting the migration of the metal is to biodegrade the organic ligand (Figure 5.5). The resulting free metal ions are likely to adsorb to mineral surfaces or form oxide mineral precipitates that would transport poorly in groundwater. A number of EDTA- and NTA-degrading organisms have been identified. However, little is known about the enzymes that catalyze the degradation reactions and how these reactions proceed in the environment. 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. Similar fundamental research focusing on the mechanisms of enzymatic degradation of synthetic chelators is expected to provide useful information for including these enzymes in engineered bioremediation technologies.

Biologically Catalyzed Redox Reactions that Lead to Solubilization

Solubilization of biosorbed and coprecipitated metals also can occur by direct or indirect microbial processes. However, the solubilization of toxic heavy metals and radionuclides from coprecipitates requires at least partial solubilization of the oxide mineral itself. Bacteria can catalyze the dissolution of iron oxide minerals by direct and indirect mechanisms. As previously described, metal-reducing bacteria enzymatically reduce and, under proper environmental conditions, solubilize oxide minerals. Such dissolution reactions have been shown to release cadmium, nickel, and zinc into solution during reduction of goethite (a form of iron oxide) by an anaerobic *Clostridium* species. Direct reduction of iron oxide precipitates by metal-reducing bacteria has been shown to release soluble radium from uranium mine tailings. Direct enzymatic reduction of iron oxides provides potential for releasing a wide range of heavy metals and radionuclides that were coprecipitated and immobilized in subsurface sediments. However, not all metals have been tested and more research is needed in this area.

Metal-reducing bacteria also can promote the mobilization of insoluble forms of some heavy metals. For example, PuO_2 exists as a solid in contaminated environments. It has been demonstrated that metal-reducing bacteria solubilized PuO_2 in the presence of the synthetic chelator NTA. It is thought that the bacteria reduced 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. This 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.

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. In some cases the organic metabolites also serve as chelating agents that can form soluble metal–ligand complexes. These chelating agents, such as 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, and copper, uranium, thorium, and nickel oxides, and can accelerate the movement of metals in soils and sediments.

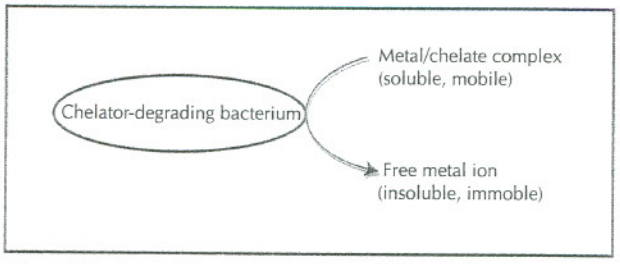

Figure 5.5. Immobilization of radionuclides and heavy metals by enzymatic degradation of organic chelators, such as EDTA and NTA.

A CASE STUDY
OF IN SITU STABILIZATION OF METALS AND RADIONUCLIDES

The primer concludes here with a case study of a hypothetical site. This study illustrates one type of contaminant problem occurring on DOE lands and presents a methodology that can be used to stabilize some of the more mobile contaminants on this site. The hypothetical site is representative of U.S. Department of Energy sites contaminated with complex mixtures of metals and radionuclides.

SCOPE OF PROBLEM

Contaminants Present: A complex contaminant mixture of uranium (U), chromium (Cr), and technetium (Tc) has entered an unconfined aquifer (an aquifer connected to the surface) as a result of nuclear fuel reprocessing and other operations at the site. Underlying a vadose zone of 10 meters from the ground surface (Figure 6.1), the aquifer is approximately 5 meters thick and consists of sandy gravels interspersed with sediments containing silts and clays. The contaminant plume, migrating at a rate of 0.3 meters per day, contains sufficient U, Cr, and Tc to be of regulatory concern. It also discharges to a river that constitutes an aquatic resource and drinking water supply. The water is aerobic (8–10 ppm oxygen), and the contam-

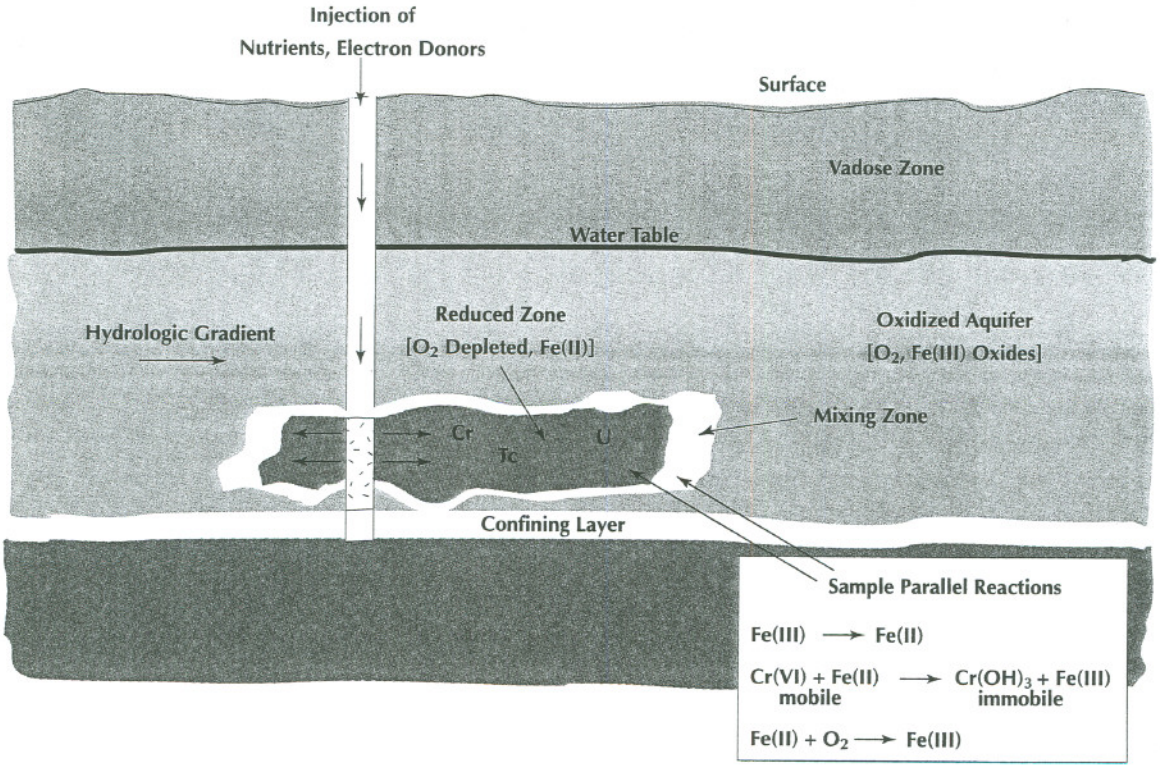

Figure 6.1. In situ stabilization of metals through accelerated bioremediation.

inants are present in oxidized states as U(VI), Cr(VI), and Tc(VII). The plume is 80 meters in width, and the site thus qualifies for immediate remedial action to protect the river.

Present Technology: The baseline technology for groundwater at the hypothetical site has been pump and treat, followed by disposal or reinjection of treated water. This process can be costly and inefficient because of difficulties in removing all of the contaminated water and contaminants sorbed on mineral surfaces. Removal and aboveground treatment of radioactive waste are also very hazardous. In addition, pump and treat can take decades and disposal of the contamination removed from the groundwater will still be necessary.

METHODOLOGY

The Alternative: Create a permeable treatment zone in the aquifer that removes the metals and radionuclides from the groundwater before they impinge on sensitive water supplies. If the groundwater is below approximately 15 meters, the treatment zone must take advantage of in situ processes, as it becomes impractical to excavate and place barrier materials below these depths.

A Role for In Situ Bioremediation: Unconfined aquifers are often oxidizing environments in which elements such as U, Cr, and Tc are mobile in their oxidized forms. Yet, microorganisms that normally operate in the absence of oxygen may occupy niches in these environments. They may also be encouraged to alter the form of these elements so that they are retained on minerals within the sediments and removed from the groundwater.

For example, a group of microorganisms known as iron reducers are able to conserve energy for growth and reproduction by converting oxidized iron [Fe(III)] to reduced iron [Fe(II)]. These organisms could directly immobilize metals and radionuclides and enzymatically convert them to chemically reduced states. The contaminants then would become associated with sediments and therefore would also become less mobile in groundwater. Or, iron reducers may indirectly immobilize these contaminants through the reduction of Fe(III) in mineral structures to Fe(II). This, in turn, chemically reduces the metals to less mobile forms. The indirect method is probably the most desirable for in situ technologies because it produces a relatively stable reactive solid phase that may exist for many years in groundwater environments, forming a long-lasting permeable barrier to further transport of the contaminants.

The Challenges: Taking advantage of native populations of microorganisms for in situ treatments to remove metals and radionuclides from groundwater is very challenging. Obstacles must be overcome by innovative science and engineering, making use of the disciplines of microbiology, geochemistry, hydrology, and geophysics. However, the potential benefits are immense because use of indigenous microorganisms may eliminate the need for pumping and treating, particularly in situations that require immediate action.

IMPLEMENTATION AND MONITORING STEPS

1. **Facilitate the growth of iron-reducing organisms:** This might be accomplished by supplying readily available organic carbon to native heterotrophic microorganisms that use up oxygen in the water, thereby supplying electron donors. Bioaugmentation could also be put into play by concentrating native organisms from the groundwater and reinoculating them into groundwater at the subsurface barrier location.

2. **Estimate biotic reduction of subsurface minerals:** This will require an understanding of the mineralogy of the subsurface and the effect of iron-reducing microorganisms on surface iron and structural iron.[1] When surface iron is reduced to Fe(II) it will be re-adsorbed into the treatment zone or the oxidized zones downgradient from the treatment zone. (In this scenario, structural iron will serve as the primary long-term reducing agent.) This step will also require an understanding of the interactions of the oxidized contaminants with biologically reduced minerals and the ability to predict the duration of immobilization under groundwater conditions.

3. **Deliver microorganisms, carbon sources, and electron donors:** Major challenges exist in creating an in situ treatment at a specific location in the subsurface, which can only be visible remotely through the narrow window of observation offered by drilling. Detailed hydrologic models coupled with geophysical, geochemical, and biological process-level information and models must be used in an integrated way to establish treatment conditions in time and space.

4. **Monitor and evaluate results:** Challenges similar to those that exist in design and implementation of the treatment process also occur in evaluating the results of treatment. The long-term effectiveness of the treatment and potential impact on natural microbial communities must be determined. Key questions that must be answered include: How effective was reduced iron in removing the contaminants? When will structural iron be completely utilized and retreatment needed? What is the likelihood that a pulse of contaminant will be released from the barrier as the system reoxidizes?

REWARDS

The fundamental knowledge needed to use biological processes for in situ treatment of metals and radionuclides and predicting the effects on groundwater systems is formidable. However, the ability to effectively stabilize contaminant movement in the subsurface with a minimum use of energy and chemicals offers a new and perhaps cost-efficient tool for situations where the existing baseline technology is not acceptable.

1. Structural iron is associated with the crystalline mineral. Amorphous iron forms a coating over the mineral. Because structural iron is an integral part of the mineral itself, it serves better as a long-term reducing agent than amorphous iron, which may be dissolved and eventually lost in the groundwater.

GLOSSARY

Absorption: The process of taking up, of absorbing or of 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).

Actinomycetes: A heterogeneous group of gram-positive, generally aerobic bacteria. They have a filamentous and branching growth pattern resulting in an extensive colony, or mycelium. The mycelium in some species may break apart to form rod or coccoid-shaped forms. Many genera also form spores.

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 groundwater.

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

Algae: Photosynthetic eukaryotic unicellular and simple multicellular microorganisms.

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

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

Anion: A negatively charged ion.

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

Archaea (formerly, archaebacteria): 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: Organism able to utilize carbon dioxide as a sole source of carbon.

Bacteria (formerly, eubacteria; singular bacterium): 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 the bodies of 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.

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

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 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.

Biosequestration: The conversion of a compound through biological processes to a form that is chemically or physically isolated or inert.

Biosorption: Sorption of a molecule by an organism.

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 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 are 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.

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: A one-sided type of symbiosis where organisms from different species live in close proximity to one another, in which the members of one are unaffected by the relationship and the members of the other benefit.

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.

Consortium: A group of organisms that interact within a given environment.

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: Protein in the cell membrane that is involved in the transfer of electrons from a substrate to a terminal electron acceptor.

Cytoplasm: Cellular contents inside the cytoplasmic membrane.

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

Denitrification: the formation of gaseous nitrogen (N_2) or nitrogen oxide (NO or N_2O) from nitrate (NO_3^-) or nitrite (NO_2^-) by microorganisms.

Dense Non-Aqueous Phase Liquid (DNAPL): Liquid contaminant that is 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 and T and between G and C. 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; the mechanical mixing of solutes that occurs as the solutes are advected through the groundwater system.

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.

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.

Extremophiles: A group of microorganisms whose growth is dependent on extreme environmental conditions.

Facultative: Used to indicate that an environmental factor is optional. For example, 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 of fungi include molds, rusts, mildews, smuts, mushrooms, and yeasts.

Gene: The fundamental unit of heredity consisting of an ordered sequence that codes for a particular polypeptide chain (molecular chain of amino acids) or RNA 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.

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

Groundwater: 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.

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.

In situ: In the original position or place.

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

Inorganic Compounds: Chemicals that do not contain carbon, which is usually associated with life processes; for example, metals are inorganic.

Insoluble: Incapable of being dissolved in a liquid.

Intrinsic Bioremediation: Bioremediation at a given site as a function of the naturally occurring microbial population and naturally occurring chemical, biological, and geological conditions. Also known as natural attenuation when dominated by biological processes, or natural bioremediation.

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).

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.

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 contaminant that is relatively insoluble and lighter than water.

Lipid: A diverse group of water-insoluble organic molecules important in the structure of the cell membrane and (in some organisms) the cell wall.

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: Oxidation of methane as a source of carbon by microorganisms known as methanotrophs.

Microbiology: A branch of biology dealing especially 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.

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 where organisms from different species live in close proximity to one another, in which all organisms involved benefit from the relationship.

Natural Attenuation: Unengineered or human-influenced degradation or transformation of contaminants in an environment via naturally occurring physical, chemical, and biological processes. May include intrinsic bioremediation.

Nucleotide: A subunit of DNA or RNA consisting of a nitrogenous base, a phosphate molecule, and a sugar molecule. Thousands of nucleotides are linked to form a DNA or RNA molecule.

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

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 groundwater 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.

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. Each unit of change (e.g., from 7 to 6) represents a tenfold change in acidity or 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.

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: Remediation influenced by eukaryotic plants.

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.

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.

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.

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: Here, chemical 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.

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 nucleic acid containing ribose and uracil as structural components. It is found in the nucleus and cytoplasm of cells and plays an important role in protein synthesis and other chemical activities of the cell. The structure of RNA is similar to that of DNA.

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.

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 Phosphorylation: Synthesis of ATP through the reaction of inorganic phosphate with an activated (usually) organic substrate. Occurs during fermentation.

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

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. The main types of microbial symbiotic relationships are mutualism, commensalism, and parasitism.

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 the 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.

ACRONYMS

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
FAME:	Fatty acid methylester
GEM:	Genetically engineered microorganism
HLW:	High-level radioactive waste
LNAPL:	Light non-aqueous phase liquid
NABIR:	Natural and Accelerated Bioremediation Research program
NAPL:	Non-aqueous phase liquid
NTA:	Nitrilotriacetic acid
PAH:	Polycyclic aromatic hydrocarbon
PLFA:	Polar lipid fatty acids
PCB:	Polychlorinated biphenyl
PCR:	Polymerase chain reaction
RNA:	Ribonucleic acid
rRNA:	Ribosomal RNA
STCGs:	Site Technology Coordination Groups
TCE:	Trichloroethylene
VOC:	Volatile organic compound

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Bioremediation Web Sites

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

BEST (Bioremediation, Education, Science and Technology):
http://bark214-3.berkeley.edu/BEST

Biological and Environmental Research Program (of the DOE Office of Science):
http://www.er.doe.gov/production/ober/ober_top.html

Bioremediation Discussion Group:
http://biogroup.gzea.com/

Bioremediation Resources on the Internet (Univ. Guelph):
http://gwrp.cciw.ca/internet/bioremediation/

Bioremediation: Nature's Way to a Cleaner Environment (USGS):
http://h2o.usgs.gov/public/wid/html/bioremed.html

Center for Biofilm Engineering:
http://www.erc.montana.edu/

Center for Environmental Biotechnology (CEB) (Univ. Tennessee, Knoxville):
http://web.utk.edu/~cebweb/cebfinal.html

Center for Hazardous Waste Remediation Research (Univ. Idaho):
http://image.fs.uidaho.edu/center2/

Clu-In (USEPA Hazardous Waste Clean-Up Information):
http://clu-in.com/

EMSL — The William R. Wiley Environmental Molecular Sciences Laboratory (a national scientific user facility):
http://www.emsl.pnl.gov

EnviroInfo, a Resource Compilation:
http://www.deb.uminho.pt/fontes/enviroinfo/enviroinfo.htm

Genome Sequence Database:
http://www.ncgr.org/gsdb/

In Situ Bioremediation, Aquarius, Vol. 23, Issue 2, May 1994:
http://publish.uwrl.usu.edu/aqmay94.html

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

MAGPIE (Automated Genome Project Investigation Environment):
http://www-fp.mcs.anl.gov/~gaasterland/magpie.html

MBI International:
http://www.mbi.org/

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

Microbial Biogeochemistry Group, Oak Ridge National Laboratory:
http://www.esd.ornl.gov/programs/microbes/

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

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

Oregon Collection of Methanogens:
http://caddis.esr.pdx.edu/OCM/

Ribosome Database Project (Michigan State Univ. Center for Microbial Ecology):
http://www.cme.msu.edu/RDP/

Site Technology Coordination Groups (STCGs):
http://em-52.em.doe.gov/ifd/stcg/stcg.htm

Subsurface Microbial Culture Collection:
http://caddis.esr.pdx.edu/smccw/

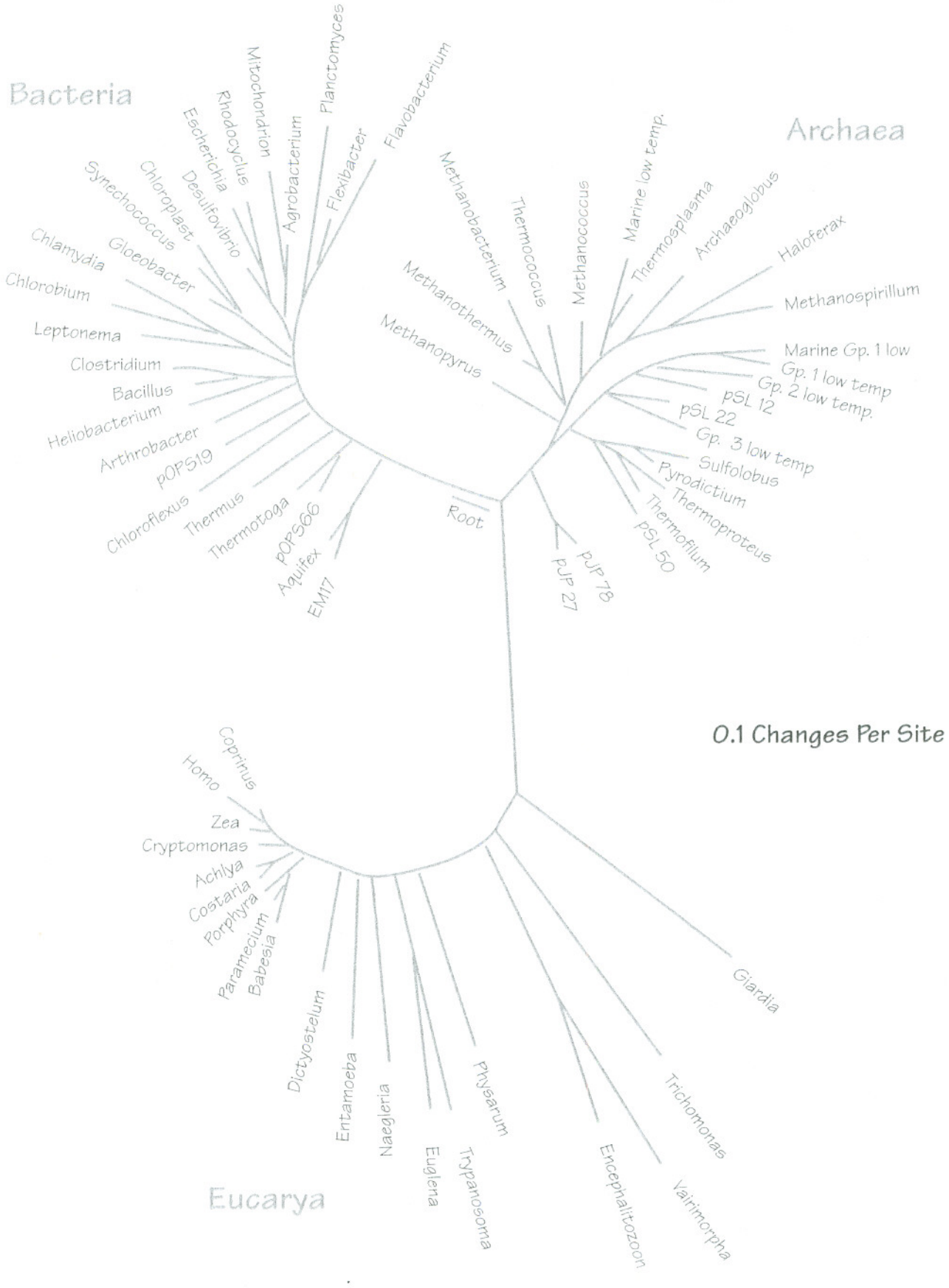
Superfund Basic Research Program:
http://www.niehs.nih.gov/sbrp/home.htm

The University of Minnesota Biocatalysis/Biodegradation Database (database of microbial biocatalytic reactions and biodegradation pathways primarily for xenobiotic, chemical compounds):
http://www.labmed.umn.edu/umbbd/

TIGR Microbial Database (Institute for Genomic Research database for microbial genomes):
http://www.tigr.org/tdb/mdb/mdb.html

U.S. Federation for Culture Collections:
http://caddis.esr.pdx.edu/usfcc/

WIT — What is There? (Interactive Metabolic Reconstruction on the Web):
http://www-c.mcs.anl.gov/home/compbio/WIT/wit.html



The Molecular Tree of Life. This expansion of Carl Woese's phylogenetic tree is by Norman Pace, University of California at Berkeley and Lawrence Berkeley National Laboratory.