Bioremediation

After completing this chapter, you should be able to:

- Explain what bioremediation is and describe why it is important.
- Describe advantages of bioremediation strategies over other types of cleanup approaches.
- Name common chemical pollutants that need to be cleaned up in different zones of the environment.
- Distinguish between aerobic and anaerobic biodegradation and provide examples of microbes that can contribute to bioremediation.
- Explain why studying genomes of organisms involved in bioremediation is an active area of research.
- Define phytoremediation and understand how it can be used to clean up the environment.
- Discuss how in situ and ex situ approaches can be used to bioremediate soil and groundwater.
- Discuss the roles of bioremediation at a wastewater treatment plant.
- Provide examples of how genetically modified organisms can be used in bioremediation.

Landfills around the world are overflowing with tons of garbage that are generated each day. Bioremediation has great potential for cleaning up chemicals in the environment, reducing waste in landfills, and even producing energy from society's garbage.

Maciej Dakowicz/Alamy.

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Our environment is being threatened with alarming frequency. The air we breathe, the water we drink, and the soil we rely on to grow plants for food are all being contaminated as a direct result of human activities. The average American generates approximately 5 pounds of garbage per day—over 1,700 pounds of trash in a year per person, leading to a total of about 250 million tons of trash every year. Only about one-third (some 83 million tons) of this trash is recycled or composted; the remainder goes into landfills. Yet household wastes are a relatively small part of the problem. Pollution from industrial manufacturing wastes as well as from chemical spills, household products, and pesticides has led to contamination of the environment. An increasing number of toxic chemicals are presenting serious threats to the health of environments throughout the world and to the organisms that live there.

Just as biotechnology is considered to be a key to identifying and solving human health problems, it is also a powerful tool for studying and correcting the poor health of polluted environments. In this chapter we consider how biotechnology can help solve some of our pollution problems and create cleaner environments for humans and wildlife through bioremediation.

FORECASTING THE FUTURE

There are many exciting potential future directions for bioremediation. Among the most likely areas of substantial progress will be global efforts in bioprospecting to find previously undiscovered metabolizing microbes and studying the genomics of these microbes to design pollution-degrading bacteria and plants. For example, in this chapter we discuss how scientists are analyzing oil-degrading microbes discovered in the Gulf of Mexico at the site of the *Deepwater Horizon* oil spill. Creating genetically engineering microbes and plants to degrade particularly toxic and harmful pollutants such as heavy metals and radioactive compounds will continue to be a focus of bioremediation, as will using bioremediation microbes as an energy source. Also, keep an eye out for applications of synthetic biology to artificially create new types of metabolizing microbes with synthetic genomes including genes customized for degrading specific pollutants.

1 What Is Bioremediation?

Bioremediation is the use of living organisms such as bacteria, fungi, and plants to break down or degrade chemical compounds in the environment. It takes advantage of natural chemical reactions and processes through which organisms break down compounds to obtain nutrients and derive energy. Bacteria, for example, metabolize sugars to make adenosine triphosphate (ATP) as an energy source for cells. But in addition to degrading natural compounds to obtain energy, many microbes have developed unique metabolic reactions that can be used to degrade human-made chemicals. Bioremediation cleans up environmental sites contaminated with pollutants by using living organisms to degrade hazardous materials into less toxic substances.

Bioremediation is not a new application. Humans have relied on biological processes to reduce waste materials for thousands of years. In the simplest sense, the outhouse—which relied on natural microbes in soil to degrade human wastes—was an example of bioremediation. Similarly, sewage treatment plants have used microbes to degrade human wastes for decades. But as you will learn in this chapter, modern applications in bioremediation involve a variety of new and innovative strategies to clean up a wide range of toxic chemicals in many different environmental settings.

Taking advantage of what many microbes already do is only one aspect of bioremediation. One of its key purposes is to improve natural mechanisms and increase rates of biodegradation to accelerate cleanup processes. In this chapter, we explore some of the ways in which scientists can stimulate microbes and other organisms to degrade a variety of wastes in many different situations. Another important aspect of bioremediation is the development of new approaches for the biodegradation of waste materials in the environment, which can involve using plants and genetically modified microorganisms.

Why Is Bioremediation Important?

Our quality of life is directly related to the cleanliness and health of the environment. We know that environmental chemicals can influence our genetics and that some chemicals can act as mutagens, leading to human disease conditions. Clearly, there is reason to be concerned about both short- and long-term chemical exposure and the effects of environmental chemicals on humans and other organisms.

According to some estimates, over 200 million tons of hazardous materials are produced in the United States each year. Accidental chemical spills can and do occur, but these events typically are contained and cleaned up rapidly to minimize their impact on the environment. More problematic, however, are illegal dumping practices and sites contaminated through neglect, such as abandoned warehouses, where stored chemicals may leak into the environment. In 1980, the U.S. Congress established the **Superfund Program** as an initiative of the **U.S. Environmental Protection Agency (EPA)** to counteract careless and even negligent practices of chemical dumping and storage as well as to express concern over how these pollutants might affect human health and the environment. The primary purpose of the Superfund Program is to locate and clean up hazardous waste sites in order to protect U.S. citizens from contaminated areas.

One in every five Americans lives within 3 to 4 miles of a polluted site treated by the EPA. In the more than 25 years since Superfund began, the EPA has cleaned up more than 700 sites in the United States. Nevertheless, well over 1,000 sites await cleanup, and Department of Energy estimates suggest as many as 220,000 sites need remediation, with many new sites identified each year. Estimates for the cleanup costs of currently identified polluted areas in the United States are in excess of $1.5 trillion. The extent of contamination of other sites and the number of sites requiring cleanup will likely push that estimate much higher. Clearly environmental pollution in the United States is an important problem that is receiving a lot of attention. In many other countries, environmental pollution is an even greater problem. To learn more about the Superfund Program, visit the Superfund website, which is listed on the Companion Website. Through this site you can also check on contaminated environments close to where you live that are on the Superfund priority list for cleanup.

Through the National Institute of Environmental Health Sciences, a division of the National Institutes of Health, the United States started a program called the **Environmental Genome Project.** A primary purpose of this project is to study and understand the impacts of environmental chemicals on human disease. This includes the study of genes that are sensitive to environmental agents, learning more about detoxification genes, and identifying single-nucleotide polymorphisms that may be indicators of environmental impacts on human health. Ultimately, this project will generate genome data enabling scientists to carry out epidemiological studies that will help them not only to better understand how the environment contributes to disease risk but also how specific diseases are influenced by environmental exposure to chemicals.

We know that pollution is a problem that can affect human health. However, there are many ways to clean up pollutants, so why use bioremediation? We could physically remove contaminated material such as soil or chemically treat polluted areas, but these processes can be very expensive and, in the case of chemical treatments, can create more pollutants that themselves require cleanup. A major advantage of bioremediation is that most such approaches convert harmful pollutants into relatively harmless materials such as carbon dioxide, chloride, water, and simple organic molecules. Because living organisms are used for the cleanup, bioremediation processes are generally cleaner than other types of cleanup strategies.

Another advantage of bioremediation is that many cleanup approaches can be conducted at the site (in situ) of pollution. Because the contaminated materials do not need to be transported to another site, a more complete cleanup is often possible without disturbing the environment. In the next section, we consider some of the basic principles of bioremediation in terms of common chemical pollutants and the environments they pollute. We also discuss some of the microbes and reactions that are important for bioremediation.

2 Bioremediation Basics

Naturally occurring marshes and wetlands have excelled at bioremediation for hundreds of years. In these environments, plant life and microbes can absorb and degrade a wide variety of chemicals and convert pollutants into harmless products. Before we discuss how living organisms can degrade pollutants, we will first consider areas of the environment that require cleanup and take a look at some of the common chemicals that pollute the environment.

What Needs to Be Cleaned Up?

Unfortunate as it may be, the answer to this question is that almost everything needs to be cleaned up. Soil, water, and sediment (a combination of soil and decaying plant and animal life located at the bottom of a body of water) are the most common treatment environments that require cleanup by bioremediation, although new bioremediation approaches are being developed to detect and clean up air pollution. Each area presents its own complexities for cleanup because the type of bioremediation approach used typically depends on site conditions. For instance, it should not surprise you that approaches for cleaning up soil can be very different from those used to clean up water. Similarly, surface water often needs to be treated differently than subsurface water (called groundwater).

Pollutants enter the environment in different ways and affect diverse components of the environment. In some cases, pollutants enter the environment through a tanker spill, a truck accident, or a ruptured chemical tank at an industrial site. Of course, depending on the location of the accident, the amount of chemicals released, and the duration of the spill (hours versus weeks or years), different parts or zones of the environment may be

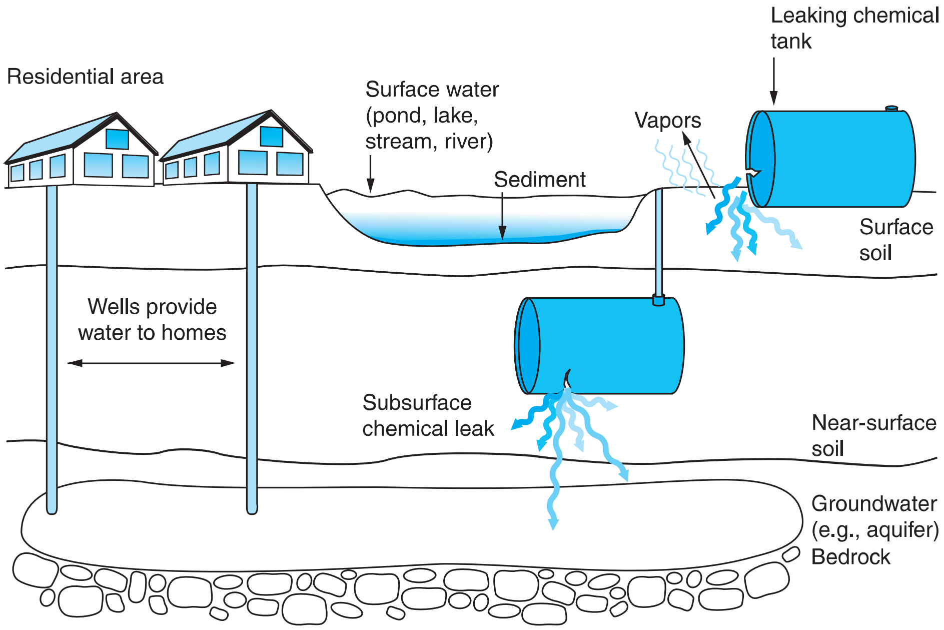

FIGURE 1 Treatment Environments and Contamination Zones Chemical spills can create a number of treatment environments and zones of contamination that may be targets for bioremediation. A spill from a leaking chemical tank, which may be located above the ground or below the surface, can release materials that contaminate surface soil, subsurface soil, surface water, and groundwater. In this example, pollution of the aquifer threatens the health of individuals living in houses adjacent to the spill, who rely on the aquifer as a source of drinking water.

affected. Figure 1 provides an example of a leaking chemical tank at an industrial plant. It may initially contaminate only surface and subsurface soils; however, if large amounts of chemicals are released and the leaky tank goes undetected for a long time, chemicals may move deeper into the soil. Following heavy rains, these same chemicals may create runoff that can contaminate adjacent surface water supplies such as ponds, lakes, streams, and rivers. Chemicals may also leak through the ground, creating **leachate**. Leachate can cause contamination of groundwater, including aquifers—deep pockets of underground water that are a common source of drinking water.

Chemicals may also enter the environment through the release of pollutants into the air, which can become trapped in clouds and contaminate surface water and then the groundwater when it rains. This is what causes acid rain, for example. Pollutants from industrial manufacturing, landfills, illegal dumps, pesticides used for agriculture, and mining processes also contribute to environmental pollution. Because the bioremediation approach used to clean up pollution depends on the treatment environment, cleaning up soil is very different from cleaning up water. How bioremediation is used also depends on the types of chemicals that need to be cleaned up.

Chemicals in the Environment

Techniques for using bioremediation to degrade human wastes at a sewage treatment plant are quite different (and somewhat simpler) than those used to degrade the variety of chemicals that exist in the environment. Everyday household materials such as cleaning agents, detergents, perfumes, caffeine, insect repellents, pesticides, fertilizers, perfumes, and medicines appear in our wastewater. Increasingly researchers are also finding that U.S. waterways contain prescription and over-the-counter drugs, including contraceptives, painkillers, antibiotics, cholesterol-lowering drugs, antidepressants, anticonvulsants, and anticancer drugs. Other chemicals that make their way into the environment are the products of industrial manufacturing processes or, as discussed earlier, the result of accidents.

Numerous chemicals from many different sources are common pollutants in the environment. Table 1 lists some of the most common categories of chemicals in our environment that require cleanup. Many of these chemicals are known to be potential mutagens and **carcinogens**—compounds that cause cancer. Although we do not discuss the health effects of chemical pollutants in any detail, most of these chemicals are known to cause illnesses ranging from skin rashes to birth defects and different types of cancer, and they can poison animal and plant life. Quite simply, the presence of pollutants in an environment leads to an overall decline of the environment along with the health of the organisms living within it.

In addition to the type of spill and the cleanup environment, the type of chemical pollutant also affects the types of cleanup organisms and approaches that can be used for bioremediation. Throughout this

TABLE 1 TWENTY OF THE MOST COMMON CHEMICAL POLLUTANTS IN THE ENVIRONMENT

Chemical Pollutant	Source
Benzene	Petroleum products used to make plastics, nylon, resins, rubber, detergents, and many other materials
Chromium	Electroplating, leather tanning, corrosion protection
Creosote	Wood preservative to prevent rotting
Cyanide	Mining processes and manufacturing of plastics and metals
Dioxin	Pulp and paper bleaching, waste incineration, and chemical manufacturing processes
Methyl t-butyl ether (MTBE)	Fuel additive, automobile exhaust, boat engines, leaking gasoline tanks
Naphthalene	Product of crude oil and petroleum
Nitriles	Rubber compounds, plastics, and oils
Perchloroethylene/ tetrachloroethylene (PCE), trichloroethene (TCE), and trichloroethane (TCA)	Dry cleaning chemicals and degreasing agents TCE is present in some 34% of U.S. water supplies and 60% of Superfund sites
Pesticides (atrazine, carbamates, chlordane, DDT) and herbicides	Chemicals used to kill insects (pesticides) and weeds (herbicides)
Phenol and related compounds (chlorophenols)	Wood preservatives, paints, glues, textiles
Polychlorinated biphenyls (PCBs)	Electrical transistors, cooling and insulating systems
Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated hydrocarbons	Incineration of wastes, automobile exhaust, oil refineries, and leaking oil from cars
Polyvinylchloride	Plastic manufacturing
Radioactive compounds	Research and medical institutions and nuclear power plants
Surfactants (detergents)	Manufacturing of paints, textiles, concrete, paper
Synthetic estrogens (ethinyl estradiol)	Female hormone (estrogen)-related compounds created by a variety of industrial manufacturing processes
Toluene	Petroleum component present in adhesive, inks, paints, cleaners, and glues
Trace metals (arsenic, cadmium, chromium, copper, lead, mercury, silver)	Car batteries and metal manufacturing processes
Trinitrotoluene (TNT)	Explosive used in building and construction industries

chapter we consider strategies for cleaning up many of the pollutants listed in Table 1.

Fundamentals of Cleanup Reactions

Microbes can convert many chemicals into harmless compounds either through **aerobic metabolism** (reactions that require oxygen [O_2]) or **anaerobic metabolism** (reactions in which oxygen is not required). Both types of processes involve *oxidation* and *reduction reactions*. You must have a basic familiarity with oxidation and reduction reactions if you are to understand biodegradation.

Oxidation and reduction reactions

Oxidation involves the removal of one or more electrons from an atom or molecule, which can change the chemical structure and properties of a molecule. In the case of a chemical pollutant, oxidation can make the chemical harmless by changing its chemical properties. Oxidation reactions often occur together with **reduction reactions**. During reduction, an atom

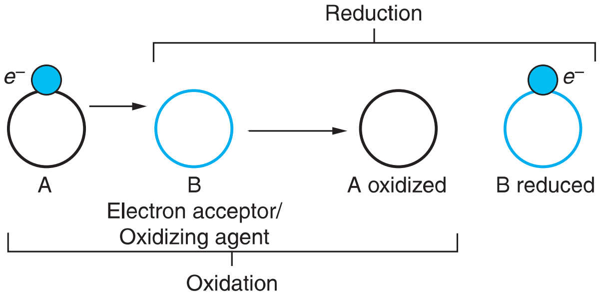

FIGURE 2 **Oxidation and Reduction (Redox) Reactions**
Redox reactions are important for the bioremediation of many chemicals. In this oxidation reaction, an electron is transferred from molecule A to molecule B. Molecule B is acting as an electron acceptor or oxidizing agent. In redox reactions, molecule A is oxidized, and molecule B, which gains an electron, is reduced.

or molecule gains one or more electrons. Because oxidation and reduction reactions frequently occur together, these electron transfer reactions are often called **redox reactions** (see **Figure 2**).During redox reactions, molecules called **oxidizing agents**—also known as electron acceptors, because they have a strong attraction for electrons—remove electrons during the transfer process. When oxidizing agents accept electrons, they become reduced. Oxygen (O_2), iron (Fe^{+3}), sulfate (SO_4^{2-}), and nitrate (NO_3^-) are often involved in redox reactions of bioremediation. Redox reactions are important for many cellular functions. For example, human cells and many other cell types use oxidation and reduction reactions to degrade sugars and derive energy.

Aerobic and anaerobic biodegradation

In some environments, such as surface water and soil where oxygen is readily available, aerobic bacteria degrade pollutants by oxidizing chemical compounds. In aerobic biodegradation reactions, O_2 can oxidize a variety of chemicals including **organic molecules** (those that contain carbon atoms), such as petroleum products (**Figure 3**). In the process, O_2 is reduced to produce water. Microbes can further degrade the oxidized organic compound to make simpler and relatively harmless molecules such as carbon dioxide (CO_2) and methane gas. Bacteria derive energy from this process, which is used to make more cells; scientists refer to this increase in cell number as an increase in **biomass**. Some aerobes also oxidize **inorganic compounds** (molecules that do not contain carbon), such as metals and ammonia.In heavily contaminated sites and deep subsurface environments such as aquifers, the concentration of oxygen may be very low. In subsurface soils, oxygen may diffuse poorly into the ground, and any oxygen that is there may be rapidly consumed by aerobes. Even though it is sometimes possible to inject oxygen into treatment sites to stimulate aerobic biodegradation in low-oxygen environments, biodegradation may take place naturally via anaerobic metabolism. Anaerobic metabolism also requires oxidation and reduction; however, anaerobic bacteria (anaerobes) rely on molecules other than oxygen as electron acceptors (**Figure 4**). Iron (Fe^{+3}), sulfate (SO_4^{-2}), and nitrate (NO_3^-) are common electron acceptors for redox reactions in anaerobes (Figures 3 and 4). In addition, some microbes can carry out both aerobic and anaerobic metabolism. When the amount of oxygen in the environment decreases, they can switch to anaerobic metabolism to continue biodegradation. As you will learn in the next section, aerobes and anaerobes are both important for bioremediation.

The Players: Metabolizing Microbes

Scientists can use microbes—especially bacteria—as tools to clean up the environment. The ability of bacteria to degrade different chemicals effectively depends on many conditions. The type of chemical, temperature, zone of contamination (water versus

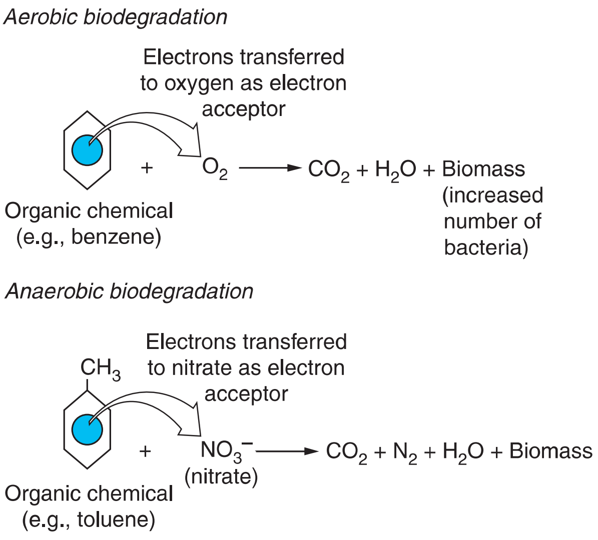

FIGURE 3 **Aerobic and Anaerobic Biodegradation**
Aerobic bacteria (aerobes) use oxygen (O_2) as an electron acceptor molecule to oxidize organic chemical pollutants such as benzene. During this process, oxygen is reduced to produce water (H_2O), and carbon dioxide (CO_2) is derived from the oxidation of benzene. Energy from degrading the pollutant is used to stimulate bacterial cell growth (biomass). Similar reactions occur during anaerobic biodegradation, except that anaerobic bacteria (anaerobes) rely on iron (Fe^{+3}), sulfate (SO_4^{2-}), nitrate (NO_3^-), and other molecules as electron acceptors to oxidize pollutants.

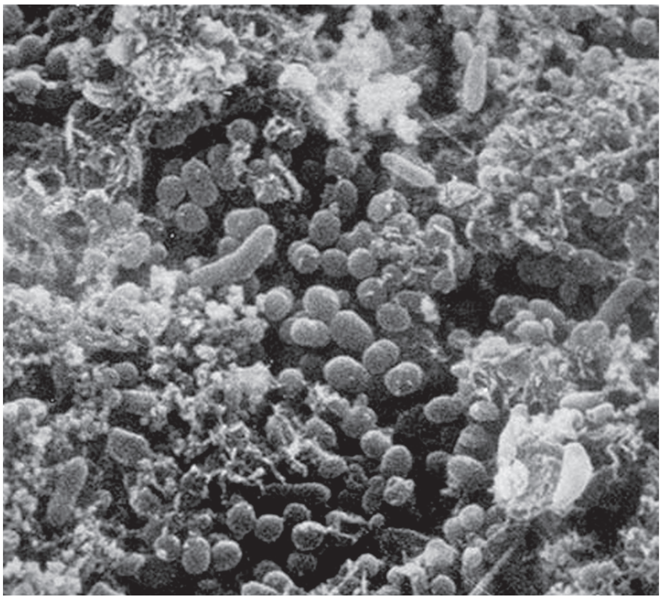

FIGURE 4 **Anaerobic Bacteria Effectively Degrade Many Pollutants** The dry-cleaning agent called perchloroethylene (PCE) is a common contaminant of groundwater; however, anaerobic bacteria can use PCE as food. By growing bacteria on small particles of iron sulfide, which serve as electron acceptors providing the proper chemical environment for anaerobes, bacteria grow rapidly and thrive on PCE.

Jennifer Bower and Ralph Mitchell.

soil, surface versus groundwater contamination, and so on), nutrients, and many other factors all influence the effectiveness and rates of biodegradation.

At many sites, bioremediation involves the combined actions of both aerobic and anaerobic bacteria to decontaminate the site fully. Anaerobes usually dominate biodegradation reactions that are closest to the source of contamination, where oxygen tends to be very scarce, but sulfates, nitrates, iron, and methane are present for use as electron acceptors by anaerobes. Farther from the source of contamination, where oxygen tends to be more abundant, aerobic bacteria are typically involved in biodegradation (**Figure 5**).

The search for useful microorganisms for bioremediation is often best carried out at polluted sites themselves. Organisms living in a polluted site will have developed some resistance to the polluting chemicals and may be useful for bioremediation. **Indigenous microbes**—those found naturally at a polluted site—are often isolated, grown, and studied in a lab and then released back into treatment environments in large numbers. Such microbes are typically the most common and effective “metabolizing” microbes for bioremediation. For instance, strains of bacteria called *Pseudomonas*, which are very abundant in most soils, are known to degrade hundreds of different chemicals. Certain strains of *Escherichia coli* are also fairly effective at degrading many pollutants.

A large number of lesser-known bacteria have been used and are currently being studied for potential roles in bioremediation. For instance, in Section 6 we discuss possible applications of *Deinococcus radiodurans*, a microbe that shows an extraordinary ability to tolerate the hazardous effects of radiation. Similarly, researchers at the University of Dublin discovered *Pseudomonas putida*, a strain of bacteria that can convert styrene, a toxic component of many plastics, into a biodegradable plastic.

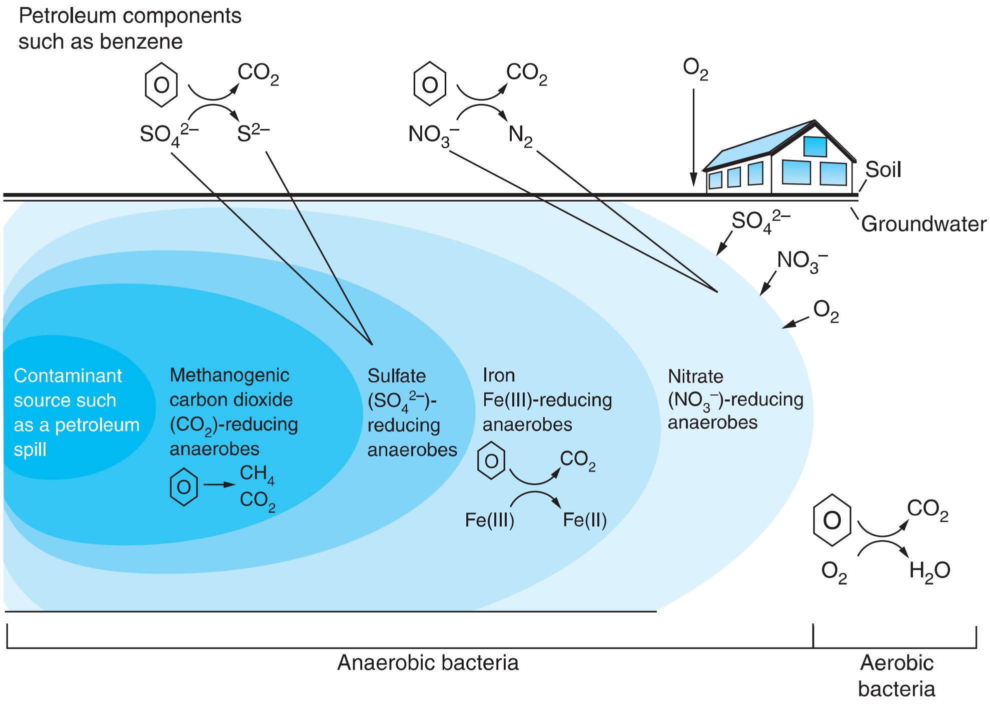

FIGURE 5 **Anaerobic and Aerobic Bacteria Contribute to Biodegradation of Contaminated Groundwater**

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Scientists believe that many of the microbes that are most effective at bioremediation have not yet been identified. The quest for new metabolizing microbes is an active area of bioremediation research. In 2010, scientists at the U.S. Geological Survey reported the identification of a bacterium from California's Lake Mono, a lake with high concentrations of arsenic, which appears to metabolize arsenic and even incorporate it into biomolecules such as DNA. Although the accuracy of discovery has been the subject of significant debate in the scientific community.

Scientists are also experimenting with strains of algae and fungi that may be capable of biodegradation. Waste-degrading fungi such as *Phanerochaete chrysosporium* and *Phanerochaete sordida* can degrade toxic chemicals such as creosote, pentachlorophenol, and other pollutants that bacteria degrade poorly or not at all. Asbestos and heavy metal–degrading fungi include *Fusarium oxysporum* and *Mortierella hyaline*. Fungi are also very valuable in composting and degrading sewage and sludge at solid-waste and wastewater treatment plants, polychlorinated biphenyls (PCBs), and other compounds previously thought to be highly resistant to biodegradation.

Bioremediation Genomics Programs

It should not surprise you to learn that many scientists are studying the genomes of organisms that are currently used or may be used in the future for bioremediation. Through genomics, it will be possible to identify novel genes and metabolic pathways that bioremediation organisms use to detoxify chemicals. This will help scientists develop more effective cleanup strategies, including improved strains of bioremediation organisms through genetic engineering (see Section 4.). It may also be possible to combine detoxifying genes from different microbes into different recombinant bacteria capable of degrading multiple contaminants at the same time—for example, PCBs and mercury.

There are a variety of ways in which molecular techniques and genomics are being used to analyze genomes of microbes at contaminated sites as a way to identify the genes and proteins involved in bioremediation. The Department of Energy established the Microbial Genome Program (MGP), which has sequenced over 200 microbial genomes, including many microbes that may be useful in bioremediation. See Table 2 for a few examples of recently completed genomics projects involving bioremediation organisms. Be sure to visit the Genomes to Life link on the Companion Website, which you can use to access the MGP and other genomics studies of bioremediation microbes.

Stimulating bioremediation

As discussed previously, bioremediation scientists typically take advantage of indigenous microbes to degrade pollutants. Depending on the pollutant, many indigenous bacteria are very effective at biodegradation. Scientists also use numerous strategies to make microorganisms more effective in degrading contaminants depending on the microorganisms involved, the environmental site being cleaned up, and the quantities and types of chemical pollutants that need to be decontaminated.

TABLE 2 EXAMPLES OF BIOREMEDIATION GENOME PROJECTS UNDER WAY OR RECENTLY COMPLETED

Microorganism	Number of Genes (Year Genome Completed)	Bioremediation Applications
Accumulibacter phosphatis	4,790 (2009)	Major microbe used in wastewater treatment plants for removing high phosphate loads from wastewaters and sludge
Alcanivorax borkumensis	2,755 (2006)	Hydrocarbon-degrading marine bacterium very effective at breaking down many components of crude and refined oil
Dehalobacter restrictus	2010 completed (details not fully available yet)	Dechlorinates perchloroethylene (PCE)
Dehalococcoides ethenogenes	1,591 (2005)	Used to degrade hydrogen and chlorine—the only known organism to fully dechlorinate perchloroethylene (PCE) and trichloroethene (TCE); PCE and TCE cleanup in wastewaters; polychlorinated dioxin degradation
Geobacter metallireducens	3,676 (2006)	Used for subsurface metal reduction, carbon cycling, and to generate electricity (see Figure 13 on page 222)
Populus trichocarpa (poplar tree)	45,555 (2006)	First tree genome sequenced; thought to have the largest number of genes of any organism sequenced to date; potentially useful for reducing atmospheric carbon dioxide

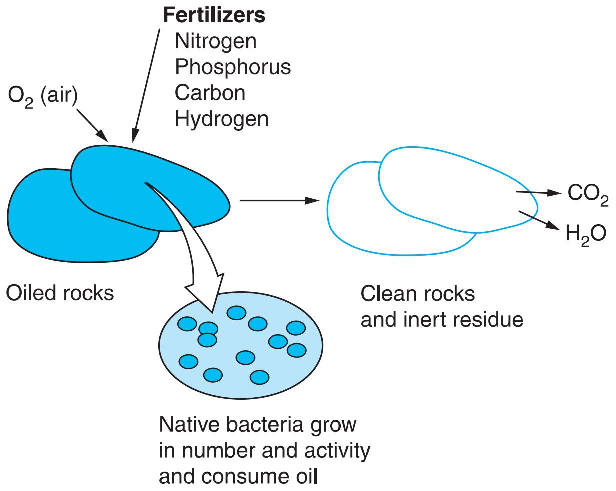

FIGURE 6 Fertilizers Can Stimulate Biodegradation by Indigenous Bacteria Bioremediation of chemicals such as those present in oil can be accelerated by adding fertilizers. Fertilizers stimulate the growth and replication of indigenous bacteria, which degrade oil into inert (harmless) compounds such as carbon dioxide (CO_2) and water (H_2O).

Nutrient enrichment, also called **fertilization**, is a bioremediation approach in which fertilizers—similar to the phosphorus and nitrogen that are applied to lawns of grass—are added to a contaminated environment to stimulate the growth of indigenous microorganisms that can degrade pollutants (**Figure 6**). Because living organisms need an abundance of key elements such as carbon, hydrogen, nitrogen, oxygen, and phosphorus for building macromolecules, adding fertilizers provides bioremediation microbes with essential elements to reproduce and thrive. In some instances manure, wood chips, and straw may be added to provide microbes with sources of carbon as a fertilizer. Fertilizers are usually delivered to the contaminated site by pumping them into groundwater or mixing them in the soil. The concept behind fertilization is simple. By adding more nutrients, microorganisms replicate, increase in number (biomass), and grow rapidly, thus increasing the rate of biodegradation.

Bioaugmentation, or **seeding**, is another approach that involves adding bacteria to the contaminated environment to assist indigenous microbes with biodegradative processes. In some cases, seeding may involve applying genetically engineered microorganisms with unique biodegradation properties. Bioaugmentation is not always an effective solution, in part because laboratory strains of microbes rarely grow and biodegrade as well as indigenous bacteria, and scientists must be sure that seeded bacteria will not alter the ecology of the environment if they persist after the contamination is gone.

Phytoremediation

A growing number of approaches are utilizing plants to clean up chemicals in the soil, water, and air in an approach called **phytoremediation.** An estimated 350 species of plants naturally take up toxic materials. Poplar and juniper trees have been successfully used in phytoremediation, as have certain grasses and alfalfa. In phytoremediation, chemical pollutants are taken in through the roots of the plant as they absorb contaminated water from the ground (**Figure 7**). As

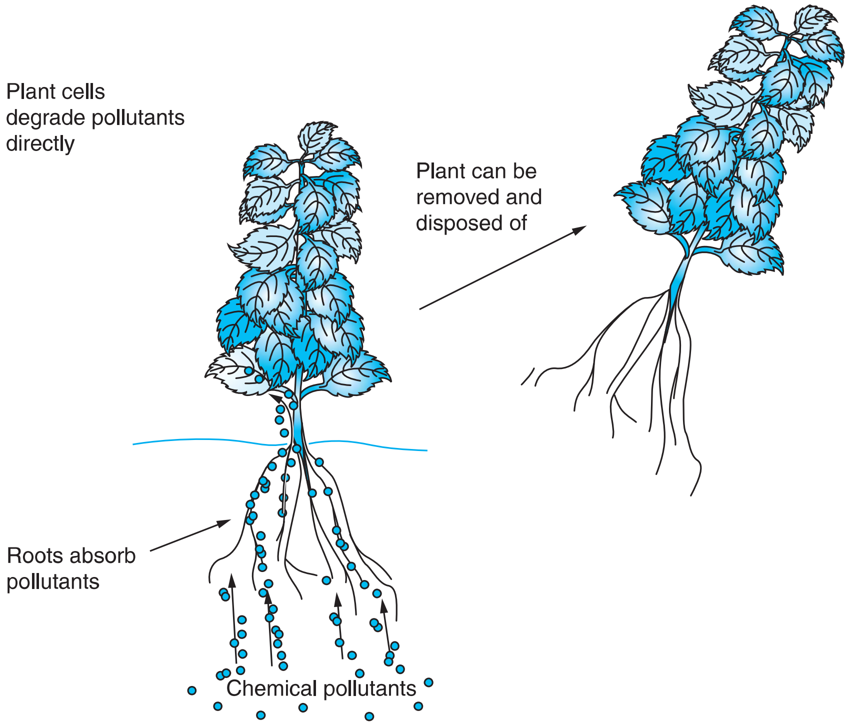

FIGURE 7 Phytoremediation Plants can be a valuable addition to many bioremediation strategies. Some plants degrade environmental pollutants directly; others simply absorb pollutants and must later be removed and disposed of.

an example, sunflower plants effectively removed radioactive cesium and strontium from ponds at the Chernobyl nuclear power plant in Ukraine. Water hyacinths have been used to remove arsenic from water supplies in Bangladesh, India. This is a significant technology, considering that arsenic concentrations exceed health standards in 60% of the groundwater in Bangladesh.After toxic chemicals enter the plant, the plant cells may use enzymes to degrade the chemicals. In other cases, the chemical concentrates in the plant cells so that the entire plant serves as a type of "plant sponge" for mopping up pollutants. The contaminated plants are treated as waste and may be burned or disposed of in other ways. Because high concentrations of pollutants often kill most plants, phytoremediation tends to work best where the amount of contamination is low—in shallow soils or groundwater.

Scientists are also exploring ways in which plants can be used to clean up pollutants in the air, something many plants do naturally: for example, removing excess carbon dioxide (CO_2), the principal greenhouse gas released from burning fossil fuels, which contributes to global warming. Recently the first genome for a tree, the black cottonwood (a type of poplar), was sequenced. Poplars are commonly used for phytoremediation, and genetically engineered poplars have shown promise for capturing high levels of CO_2.

Phytoremediation can be an effective, low-cost, and low-maintenance approach for bioremediation. As an added benefit, phytoremediation can also be a less obvious and more eye-appealing strategy. For instance, planting trees and bushes can visually improve the appearance of a polluted landscape and clean the environment at the same time. Two main drawbacks of phytoremediation are that only surface layers (to around 50 cm deep) can be treated, and cleanup typically takes several years. In the next section, we examine specific cleanup environments and different strategies used for bioremediation.

3 Cleanup Sites and Strategies

A variety of bioremediation treatment strategies exist. Which strategy is employed depends on many factors. Of primary consideration are the types of chemicals involved, the treatment environment, and the size of the area to be cleaned up. Consequently, some of the following questions must be considered before starting the cleanup process:

- Do the chemicals pose a fire or explosive hazard?
- Do the chemicals pose a threat to human health including the health of cleanup workers?
- Was the chemical released into the environment through a single incident, or was there long-term leakage from a storage container?
- Where did the contamination occur?
- Is the contaminated area at the surface of the soil? Below the ground? Does it affect water?
- How large is the contaminated area?

Answering these questions often requires the combined talents of molecular biologists, environmental engineers, chemists, and other scientists who work together to develop and implement plans to clean up environmental pollutants.

Soil Cleanup

Treatment strategies for both soil and water usually involve either removing chemical materials from the contaminated site to another location for treatment, an approach known as **ex situ bioremediation**, or cleaning up at the contaminated site without excavation or removal called **in situ** (a Latin term that means "in place") **bioremediation**. In situ bioremediation is often the preferred method of bioremediation, in part because it is usually less expensive than ex situ approaches. Also, because the soil or water does not have to be excavated or pumped out of the site, larger areas of contaminated soil can be treated at one time. In situ approaches rely on stimulating microorganisms in the contaminated soil or water. Those in situ approaches that require aerobic degradation methods often involve **bioventing**, or pumping either air or hydrogen peroxide (H_2O_2) into the contaminated soil. Hydrogen peroxide is frequently used because it is easily degraded into water and oxygen to provide microbes with a source of oxygen. Fertilizers may also be added to the soil through bioventing to stimulate the growth and degrading activities of indigenous bacteria.

In situ bioremediation is not always the best solution. This approach is most effective in sandy soils, which are less compact and allow microorganisms and fertilizing materials to spread rapidly. Solid clay and dense rocky soils are not typically good sites for in situ bioremediation, and contamination with chemicals that persist for long periods of time can take years to clean up in this way.

For some soil cleanup sites, ex situ bioremediation can be faster and more effective than in situ approaches. As shown in Figure 8, ex situ bioremediation of soil can involve several different techniques, depending on the type and amount of soil to be treated and the chemicals to be cleaned up. One common ex situ technique is called **slurry-phase bioremediation**. This approach involves moving contaminated soil to another site and then mixing the soil with water

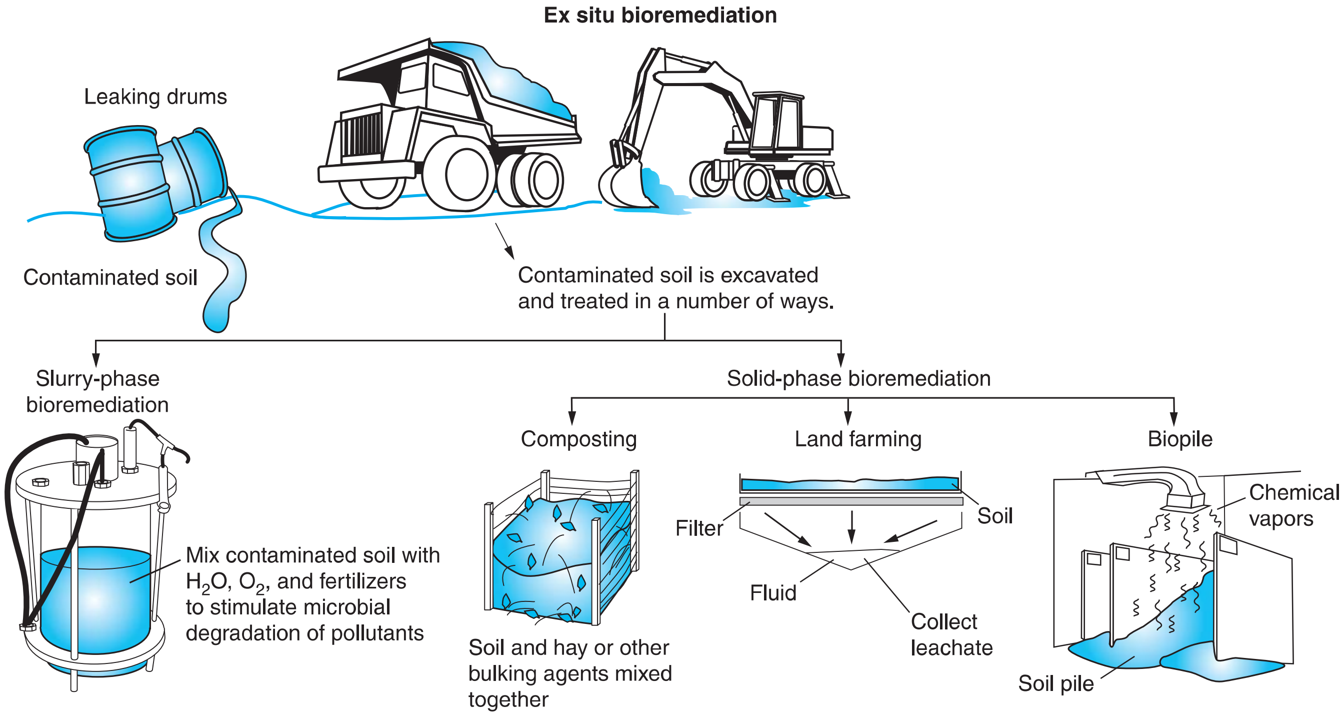

FIGURE 8 **Ex Situ Bioremediation Strategies for Soil Cleanup** Many soil cleanup approaches involve ex situ bioremediation, in which contaminated soil is removed and then subjected to several different cleanup approaches.

and fertilizers (oxygen is often also added) in large bioreactors, where the conditions of biodegradation by microorganisms in the soil can be carefully monitored and controlled. Slurry-phase bioremediation is a rapid process that works fairly well when small amounts of soil need to be cleaned and the composition of chemical pollutants is well known (Figure 8).

For many other soil cleanup strategies, **solid-phase bioremediation** techniques are required. Solid-phase processes are more time-consuming than slurry-phase approaches and typically require large amounts of space; however, they are often the best strategies for degrading certain chemicals. Three solid-phase techniques are widely used: composting, landfarming, and biopiles.

Composting can be used to degrade household wastes such as food scraps and grass clippings; similar approaches are used to degrade chemical pollutants in contaminated soil. In a compost pile, hay, straw, or other materials are added to the soil to provide bacteria with nutrients that help bacteria degrade chemicals. **Landfarming** strategies involve spreading contaminated soils on a pad so that water and leachates can leak out of the soil. A primary goal of this approach is to collect leachate so that polluted water cannot further contaminate the environment. Because the polluted soil is spread out in a thinner layer than it would be if it were below the ground, landfarming also allows chemicals to vaporize from the soil and aerates the soil so that microbes can better degrade pollutants.

Soil **biopiles** are used particularly when the chemicals in the soil are known to evaporate easily and microbes in the soil pile are rapidly degrading the pollutants (**Figure 9**). In this approach, contaminated soil is piled up several meters high. Biopiles differ from compost piles in that relatively few bulking

FIGURE 9 **Soil Biopiles** Polluted soil that has been removed from the cleanup site can be stored in piles and bioremediation processes monitored to ensure decontamination of the soil before determining whether the soil can be returned to the environment.

RJH/Alamy.

FIGURE 10 **Septic Tank Additives Stimulate Bioremediation of Household Wastes**

Michael A. Palladino.

agents are added to the soil and fans and piping systems are used to pump air into or over the pile. As chemicals in the pile evaporate, the vacuum airflow pulls the chemical vapors away from the pile and either releases them into the atmosphere or traps them in filters for disposal, depending on the type of chemical. Almost all ex situ strategies for cleaning up soil involve tilling and mixing soil to disperse nutrients, oxygenating the dirt, and increasing interaction of microbes with contaminated materials to increase biodegradation.

Bioremediation of Water

Contaminated water presents a number of challenges. In Section 5, we consider how surface water can be treated following large spills such as an oil spill. Wastewater and groundwater can be treated in many different ways, depending on the pollutants that need to be removed.

Wastewater treatment

Probably the best-known application of bioremediation is in the treatment of wastewater to remove human sewage (fecal material and paper wastes), soaps, detergents, and other household chemicals. Both septic systems and municipal wastewater treatment plants rely on bioremediation. In a typical septic system, human sewage and wastewater from a single household move through the plumbing system out to a septic tank buried below ground next to the house. In the tank, solid materials such as feces and paper wastes settle to the bottom to be degraded by microbes, while liquids flow out of the top of the tank and are dispersed underground across an area of soil and gravel called the septic bed. Within the bed, indigenous microbes degrade waste components in the water.

One commercial application of bioremediation recommended to prevent septic tanks from becoming clogged is to add products such as Rid-X (**Figure 10**), which are flushed into the system periodically. These products contain freeze-dried bacteria that are rich in enzymes such as lipases, proteases, amylases, and cellulases, which in turn degrade fats, proteins, sugars, and cellulose in papers and vegetable matter, respectively. Adding microbes this way is an example of the bioaugmentation we discussed in the previous section, and this treatment helps the septic system degrade cooking fats and oils, human wastes, tissue paper products, and other materials that can clog the system.

Wastewater (sewage) treatment plants are fairly complex and well-organized operations (**Figure 11**). Water from households that enters sewer lines is pumped into a treatment facility, where feces and paper products are ground and filtered into smaller particles, which settle out into tanks to create a mud-like material called **sludge**. Water flowing out of these tanks is called **effluent**. Effluent is sent to aerating tanks, where aerobic bacteria and other microbes oxidize organic materials in the effluent. In these tanks, water is sprayed over rocks or plastic covered with biofilms of waste-degrading microbes that actively degrade organic materials in the water.

Alternatively, effluent is passed into activated sludge systems—tanks that contain large numbers of waste-degrading microbes grown in carefully controlled environments. Usually these microbes float freely within the water, but in some cases they may be grown on filters through which contaminated water flows. Eventually effluent is disinfected with a chlorine treatment before the water is released back into rivers or oceans.

Sludge is pumped into anaerobic digester tanks in which anaerobic bacteria further degrade it. Methane-producing and carbon dioxide gas–producing bacteria are common in these tanks. Methane gas is often collected and used as fuel to run equipment at the sewage treatment plant. Tiny worms, which are usually present in the sludge, also help break down the sludge into small particles. Sludge is never fully broken down, but once the toxic materials have been removed, it is dried and can be used as landfill or fertilizer.

Scientists have discovered a bacterium called *"Candidatus Brocadia anammoxidans"* that possess a unique ability to degrade ammonium, a major waste

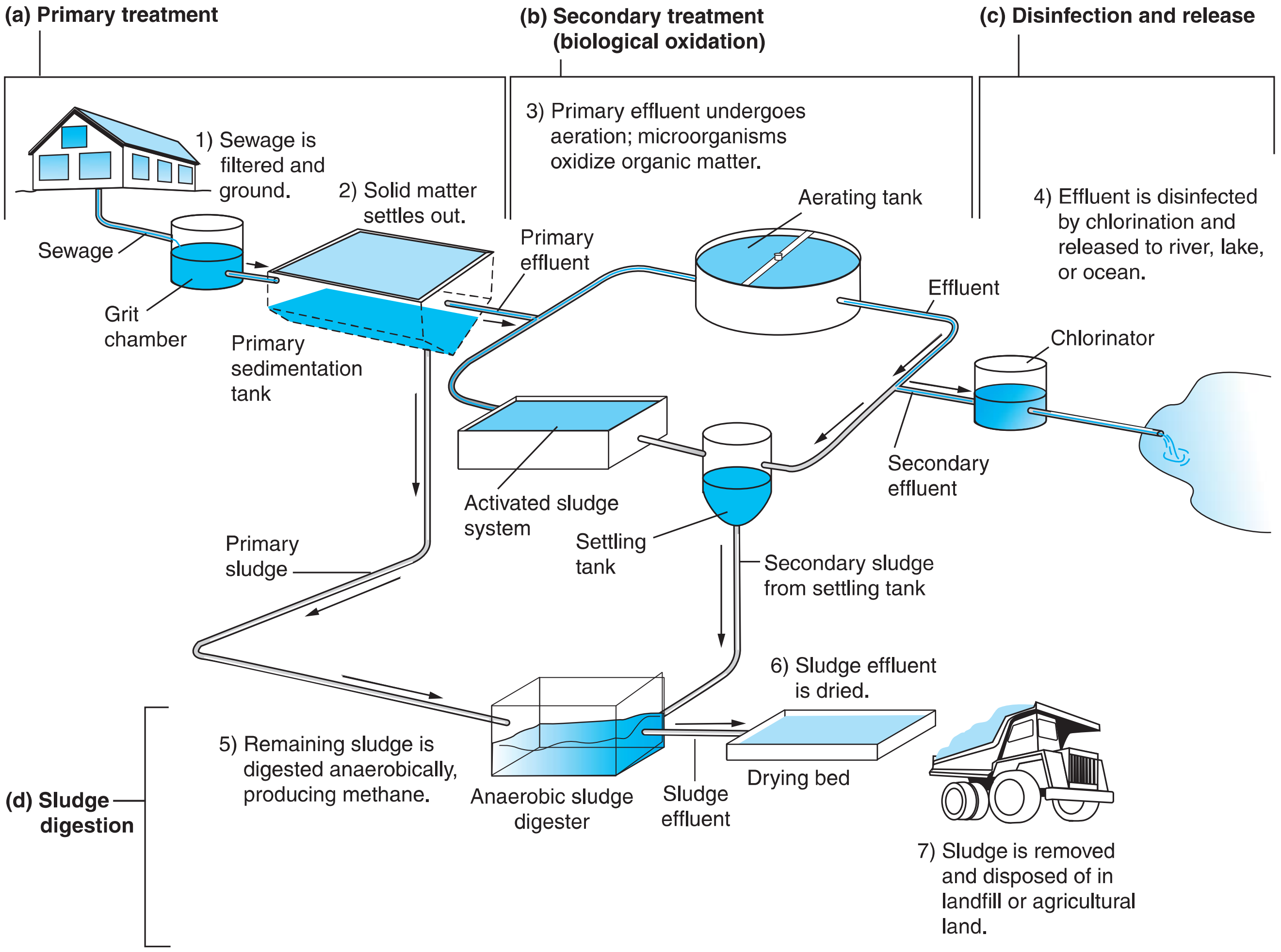

FIGURE 11 **Wastewater Treatment** Wastewater or sewage treatment facilities are well-planned operations that use aerobic and anaerobic bacteria to degrade organic materials such as human feces and household detergents in both the sludge and water (effluent).

product present in urine (**Figure 12** on the next page). Removing ammonium from wastewater before the water is released back into the environment is important because high amounts of ammonium can affect the environment by causing algal blooms and diminishing oxygen concentrations in waterways. Typically, wastewater plants rely on aerobic bacteria such as *Nitrosomonas europaea* to oxidize ammonium in a multistep set of reactions. However, *"Candidatus Brocadia anammoxidans"* is capable of degrading ammonium in a single step under anaerobic conditions, a process called the anammox process. Wastewater treatment plants in the Netherlands are using this strain and treatment plants in other countries may soon be using *"Candidatus Brocadia anammoxidans"* to remove ammonium from wastewater more efficiently.

Groundwater cleanup

With the exception of spills near coastal beaches, most large chemical spills such as oil spills occur in marine environments, often far away from populated areas. However, freshwater pollution typically occurs closer to populated areas and poses a serious threat to human health by contaminating sources of drinking water, either groundwater or surface water such as reservoirs. Groundwater contamination is a common problem in many areas of the United States. Drinking water for approximately 50% of the U.S. population comes from groundwater sources, and, according to some estimates, a large percentage of groundwater supplies in the United States contain pollutants that may have an impact on human health. Polluted groundwater can sometimes be very difficult to clean up because contaminated water gets trapped in soil and rocks and there is no easy way to "wash" aquifers.

Ex situ and in situ approaches are often used in combination. For instance, when groundwater is contaminated by oil or gasoline, these pollutants rise to the surface of the aquifer. Some of this oil or gas can be directly pumped out, but the portion mixed with groundwater must be pumped to the surface and

FIGURE 12 **Bioreactor Containing *Candidatus Brocadia anammoxidans*, Anaerobic Bacterium That Can Degrade Ammonium** Novel metabolic properties enable these anaerobes to degrade ammonium from wastewater in a single step.

ASM Publications.

passed through a bioreactor (**Figure 13**). Inside the bioreactor, bacteria in biofilms growing over a screen or mesh degrade the pollutants. Fertilizers and oxygen are often added to the bioreactor. Clean water from the bioreactor containing fertilizer, bacteria, and oxygen is pumped back into the aquifer for in situ bioremediation (Figure 13).

Turning Wastes into Energy

Landfills around the world are stressed to their limits, literally overflowing with trash from homes and businesses. The bulk of our household trash consists of food scraps, boxes, paper waste, cardboard packing containers from food, and similar items. A variety of chemical wastes such as detergents, cleaning fluids, paints, nail polish, and varnishes also make their way into the trash, despite the fact that most states are trying to reduce the amount of chemical waste that can be disposed of as regular garbage.

Scientists are working on strategies to reduce waste, including bioreactors containing anaerobic bacteria that can convert food waste and other trash into soil nutrients and methane gas. Methane gas can be used to produce electricity, and soil nutrients can be sold commercially as fertilizer for use by farms, nurseries, and other agricultural industries. Scientists are also working on seeding strategies that may be used to reduce chemicals in landfills—chemicals which would otherwise seep through the ground and contaminate local ground and surface waters. If successful, these applications of biotechnology may help to reduce the amount of waste and greatly increase the usable space in many landfills.

Bioremediation scientists are also studying polluted sediments in sewage sludge and at the bottoms of oceans and lakes as an untapped source of energy. Sediment in lakes and oceans is rich in organic materials from the breakdown of decaying materials such as

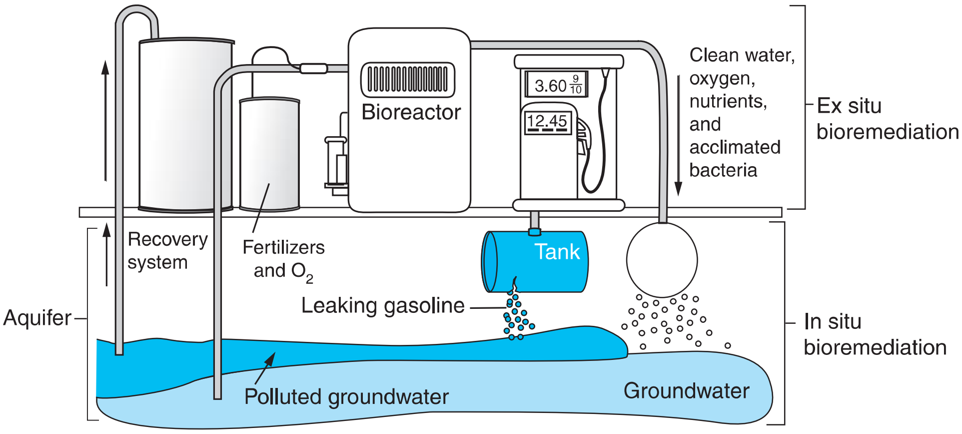

FIGURE 13 **Ex situ and in situ Bioremediation of Groundwater** Gasoline leaking into an underground water supply can be cleaned up using an aboveground (ex situ) system in combination with in situ bioremediation.

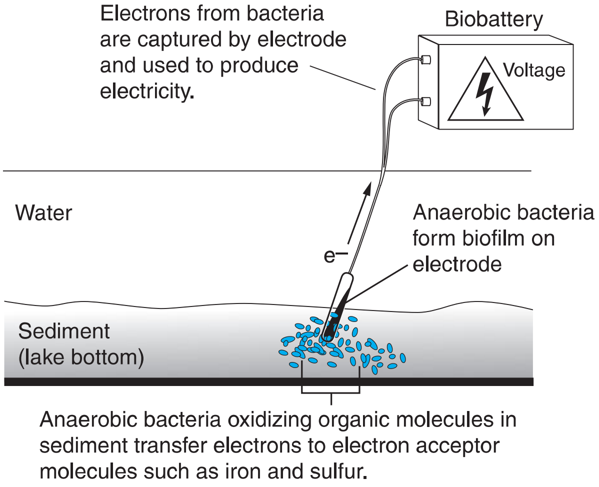

FIGURE 14 Polluted Sediments May Be an Untapped Source of Energy Scientists have found that anaerobic bacteria in sediment may be a source of energy. Because these bacteria use redox reactions to degrade molecules in sediment, electrons can be captured by electrodes, which can transfer electrons to generators to create electricity.

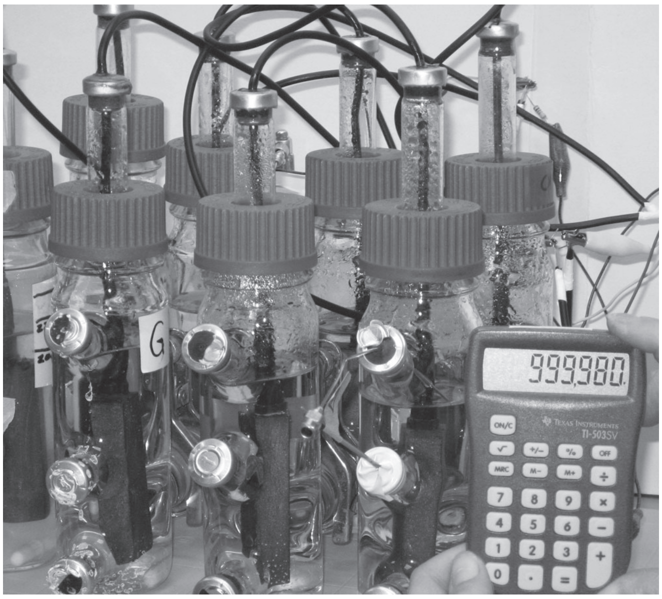

FIGURE 15 Microbe-Powered Fuel Cells Researchers at the University of Massachusetts have demonstrated that *Geobacter* fuel cells can effectively convert sugars into electricity.

Kelly P. Nevin.

leaves and dead organisms. Within this "muck" are anaerobes that use organic molecules in the sediment to generate energy. The term *electrigens* is being used to describe electricity-generating microbes that have the ability to oxidize organic compounds to carbon dioxide and transfer electrons to electrodes. Under certain conditions, electrigens can cluster and interconnect to form nanowires that conduct electrons! Such strains may even be used to make electricity from manure and common household wastes.

Desulfuromonas acetoxidans is an anaerobic marine bacterium that uses iron as an electron acceptor to oxidize organic molecules in sediment. Researchers are exploring ways in which electrons can be harvested from *D. acetoxidans* and other bacteria such as *Geobacter metallireducens* and *Rhodoferax ferrireducens* as a technique for capturing energy in *bacterial biobatteries*, also called *microbial fuel cells*, that can be used to provide a source of electricity (**Figure 14**). Researchers at the University of Massachusetts have demonstrated that electrigenic strains of microbes can generate electricity with high efficiency; although preliminary studies suggest that this technique has some promise, more research needs to be done (**Figure 15**).

Even though bioremediation strategies have effectively cleaned up many environmental pollutants, bioremediation is not the solution for all polluted sites. For instance, bioremediation is ineffective when the polluted environment contains high concentrations of very toxic substances such as heavy metals, radioactive compounds, and chlorine-rich organic molecules, because these compounds typically kill microbes. Therefore new strategies will have to be discovered and applied to tackle some of these cleanup challenges. Some of these new bioremediation strategies are likely to involve genetically engineered microorganisms, the topic of the next section.

4 Applying Genetically Engineered Strains to Clean Up the Environment

Bioremediation has traditionally relied on stimulating naturally occurring microorganisms; however, many indigenous microbes cannot degrade certain types of chemicals, especially very toxic compounds. For example, some organic chemicals produced during the manufacturing of plastics and resins are resistant to biodegradation and can persist in the environment for several hundred years. Many radioactive compounds also kill microbes, thus preventing biodegradation. To clean up some of these stubborn and particularly toxic pollutants, we may need to use bacteria and plants that have been genetically altered. The development of recombinant DNA technologies has enabled scientists to create genetically modified (GM) organisms with the potential to improve bioremediation processes.

Petroleum-Eating Bacteria

The first effective GM microbes for use in bioremediation were created in the 1970s by Ananda Chakrabarty and his colleagues at General Electric. This work was carried out before DNA cloning and recombinant DNA technologies were widely available. So how did Chakrabarty do this? He isolated strains of *Pseudomonas* from soils contaminated with different types of chemicals, including pesticides and crude oil. He then identified strains that showed the ability to degrade such organic compounds as naphthalene, octane, and xylene. Most of these strains could grow in the presence of these compounds because they contained plasmids that encoded genes for breaking down each component.

Chakrabarty mated these different strains and eventually produced a strain that contained several different plasmids. Together, the combined proteins produced by these plasmids could effectively degrade many of the chemical components of crude oil. For his work, Chakrabarty was awarded the first U.S. patent for a GM living organism. However, this patent decision was very controversial and was held up in the courts for about 10 years. The primary issues being debated were whether life forms could be patented and whether Chakrabarty's recombinant bacterium should be considered a product of nature or an invention. Eventually the U.S. Supreme Court ruled that the development of recombinant *Pseudomonas* was an invention worthy of a patent.

Chakrabarty's approach was not as effective as it might have seemed. Crude oil contains thousands of compounds, and his GM bacteria could degrade only a few of these. The majority of the chemicals in crude oil remain largely unaffected by recombinant organisms. Consequently, developing GM bacteria with different degradative properties is an intense area of research. In the future, a useful approach for cleaning up crude oil may be to release multiple bacterial strains, each with the ability to degrade different compounds in the oil. To date, field applications of GM microbes for bioremediation have been fairly limited, in part because of regulatory obstacles and public concerns over the release of GM bacteria. But GM microorganisms are also often ineffective in the environment because indigenous microbes may outcompete them.

Engineering *E. coli* to Clean Up Heavy Metals

Heavy metals including copper, lead, cadmium, chromium, and mercury can critically harm humans and wildlife. Mercury is an extremely toxic metal that can contaminate the environment. It is used in manufacturing plants, batteries, electrical switches, medical instruments, and many other products. Mercury, and a related compound called methylmercury (MeHg), can accumulate in organisms through a process called **bioaccumulation**. In bioaccumulation, organisms higher up on the food chain contain higher concentrations of chemicals than organisms lower on the food chain. For instance, in a water supply, mercury may be ingested by small fish, which may then be eaten by birds, larger fish, otters, raccoons, and other animals, including humans. Large fish and birds need to eat a lot of small fish; therefore they accumulate more mercury in their systems than small fish and birds that eat less. Similarly, if a person were to eat large fish as a primary source of food, that person would accumulate high amounts of mercury over time. Regular consumption of fish and shellfish contaminated with mercury and methylmercury poses serious health threats to humans, including birth defects and brain damage. For these reasons, in many areas of the United States, health officials suggest that pregnant women and young children eat only small amounts of certain types of fish, such as swordfish and fresh tuna, and restrict these meals to no more than one serving a week.

Because mercury is toxic at very low doses, most current strategies for removing mercury from contaminated water supplies do not remove enough of it to meet acceptable standards. Scientists have developed genetically engineered strains of *E. coli* that may be useful for cleaning up mercury and other heavy metals. They have also identified naturally occurring metal-binding proteins in plants and other organisms. Two of the best-characterized types of proteins—metallothioneins and phytochelatins—have a high capacity for binding to metals. For these proteins to function, however, the metals must enter cells. Scientists have engineered *E. coli* to express transport proteins that allow for the rapid uptake of mercury into the bacterial cell's cytoplasm, where the mercury can bind to metal-binding proteins.

Some of these genetically altered bacteria can absorb mercury directly; others that bind mercury can be grown on *biofilms* to act as sponges for soaking up mercury from a water supply. The biofilms must be changed periodically to remove mercury-containing bacteria. Similarly, genetically engineered single-celled algae containing metallothionein genes and bacteria called cyanobacteria have shown promise for their ability to absorb cadmium, another very toxic heavy metal known to cause many serious health problems in humans.

TOOLS OF THE TRADE

Microcosms Provide Major Benefits

Bioremediation scientists are working on innovative ways to biodegrade different chemical compounds under a myriad of environmental conditions. Industry is continually creating new kinds of chemicals, and many of these will inevitably make their way into the environment. To stay one step ahead of new environmental pollutants, bioremediation researchers must continue to develop new cleanup strategies.

How can scientists study bioremediation of a new chemical that has never made it into the environment? They obviously cannot pollute large areas nor wait for a large-scale disaster like the *Exxon Valdez* spill before testing their theories about how to clean up this new chemical. One of the most practical approaches to learning about new bioremediation strategies is to make a **microcosm,** an artificially constructed test environment designed to mimic real-life environmental circumstances. Some microcosms consist of small bioreactors—about the size of a 5-gallon bucket—containing soil, water, pollutants, and microbes to be tested for their bioremediation abilities. A microcosm may be as small as a few grams of soil in a test tube or vial, but they are more often scaled up to resemble larger environments. For example, large ponds, which may be indoors or outdoors, or soil plots that are prepared to prevent the escape of pollutants outside of the test facility, can be used as microcosms. By carefully designing microcosms, bioremediation researchers can attempt to simulate, on a small scale, a site that needs to be cleaned up.

When indigenous or genetically engineered organisms with bioremediation potential have been identified, studies in bioreactors or on small isolated areas of land or water can be crucial for determining whether these organisms will effectively clean up pollution in larger settings. By carefully manipulating the environmental conditions in the microcosms, scientists can test the ability of organisms to degrade different pollutants under varying conditions, including different levels of moisture, temperature, nutrients, oxygen, and pH and in different soil types.

Microcosm studies may even involve testing experimental microbes on polluted groundwater or contaminated soil that is placed into the microcosm so that the rates of degradation can be monitored and the cleanup time evaluated. Scientists can also carry out experiments to study the bioremediation of mixtures of pollutants at the same time.

In an attempt to produce the best cleanup results, scientists will analyze the data they have gathered and design new experiments. A cleanup approach that demonstrates success in a microcosm is not guaranteed to succeed in the field. Nevertheless, by testing bioremediation approaches in microcosms, much valuable time and money can be saved before deciding whether a cleanup approach is likely to have any chance of success in cleaning up a polluted environment in the field.

Biosensors

Researchers have developed genetically engineered strains of the bacterium *Pseudomonas fluorescens,* which can effectively degrade complex structures of carbon and hydrogen called polycyclic aromatic hydrocarbons (PAHs) and other toxic chemicals. Using recombinant DNA technology, scientists have been able to splice bacterial genes encoding specific enzymes (which can metabolize these contaminants) to reporter genes such as the *lux* genes from bioluminescent marine bacteria. Recall that *lux* genes are often used as reporter genes because they encode the light-releasing enzyme luciferase. As PAHs are degraded, bacteria release light that can be used to monitor biodegradation rates. Similar techniques are being used to develop **biosensors** from recombinant bacteria containing *lux* genes. Such biosensors have proven to be valuable in the assessment of environmental pollutants such as heavy metals. In the future, GM microbes are expected to play a greater role as biosensors. In the next section, we consider two of the best-studied and most highly publicized examples of bioremediation in action.

Genetically Modified Plants and Phytoremediation

In recent years scientists have been working on genetically modifying plants to improve their phytoremediation capabilities. Currently, using phytoremediation to clean up methylmercury (MeHg) is a very active area of research. Mercury contamination of most fish is a result of MeHg accumulation; throughout the world this has caused many health warnings and restrictions on consuming mercury-contaminated fish. Plants engineered to contain mercury-detoxifying genes from bacteria have shown some potential for accumulating MeHG; eventually they may be used for phytoremediation.

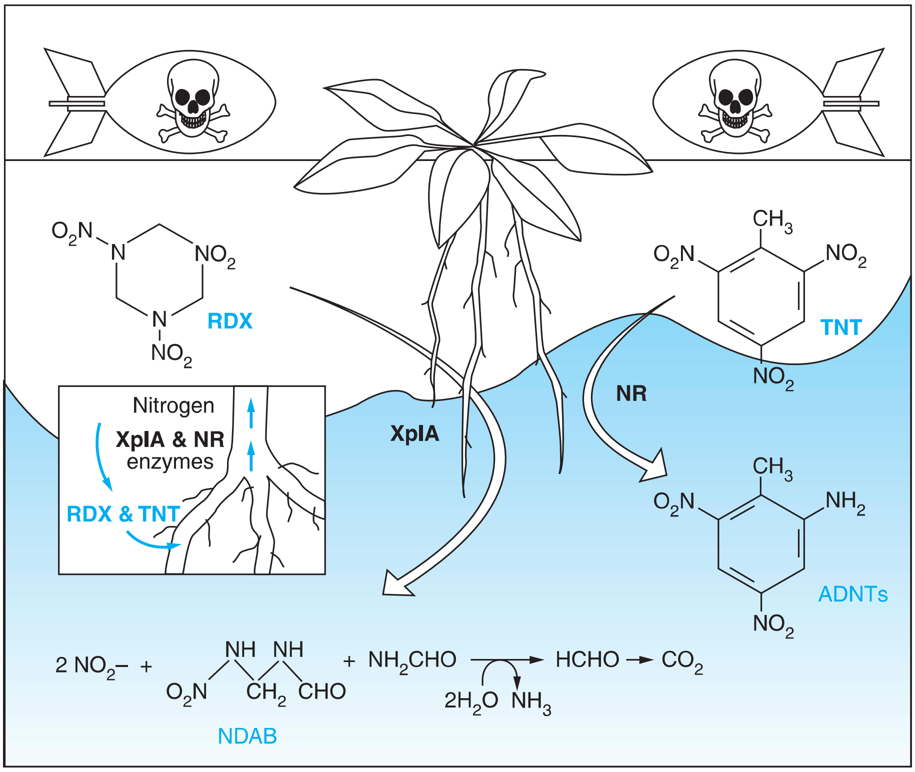

FIGURE 16 Phytoremediation of Toxic Explosives Using Transgenic Plants Transgenic plants engineered with the *XplA* or *NR* gene to degrade either RDX or TNT, respectively, absorb the explosive chemicals through their roots and then degrade them into less toxic compounds (aminodinitrotoluenes [ADNTs], or 4-nitro-2,4-diazabutanal [NDAB]), or nontoxic compounds (NH_3, CO_2). Plants engineered with the *XplA* gene use the degradation products from RDX to stimulate their growth.

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Another promising area of this research has been the development of plants able to remove chemicals from military explosives and weapons firing ranges that have contaminated soil and groundwater. Hexahydro-1,3,4-trinitro-1,3,5-triazine (commonly called royal demolition explosive, or RDX) and 2,4,6-trinitrotoluene (TNT) are two of the most common chemical contaminants that result from the production, use, and disposal of explosives. These chemicals move readily through soils and contaminate groundwater. Both RDX and TNT are highly toxic to most organisms and pose significant health threats to wildlife and humans.

Notice that TNT was one of the top 20 chemicals listed in Table 1 and that the EPA lists both TNT and RDX as priority chemicals to be removed from the environment. These contaminants are a major pollution problem worldwide. Incredibly, there are hundreds of tons of these compounds in sites around the world and tens of thousands of acres deemed unsafe as a result.

A few bacteria and plants have been shown to weakly degrade TNT with low efficiency; degradation of RDX is even less effective because of its chemical structure. In the last few years, however, scientists have used genetic engineering to create transgenic plants that may turn out to be very effective for the phytoremediation of TNT and RDX. A transgenic strain of tobacco containing a nitroreductase gene from *Enterobacter cloacae* effectively converts TNT to less toxic chemicals (**Figure 16**). Most recently, scientists incorporated a gene called *xplA* from the bacteria *Rhodococcus rhodochrous* into *Arabidopsis thaliana*. The *xplA* gene produces an RDX-degrading enzyme called cytochrome P450, which can degrade RDS once it is absorbed into the plant (see Figure 16). It may now be possible to make plants that can degrade both TNT and RDX. Moreover, since the poplar genome has been sequenced, bioremediation scientists working on genetically modified plants are very enthusiastic about the possibility of making transgenic poplars and other fast-growing, deep-rooted trees that can remediate TNT and RDX well below the surface soil. In the next section, we consider several of the best-studied and most highly publicized examples of bioremediation in action.

5 Environmental Disasters: Case Studies in Bioremediation

Most bioremediation approaches involve the cleanup of contaminated areas that are relatively small, perhaps several hundred acres in size. Nevertheless, a great deal has been learned about the effectiveness of different bioremediation strategies by studying large-scale environmental disasters that have been treated by bioremediation.

The *Exxon Valdez* Oil Spill

The world relies heavily on crude oil and on petroleum products that can be manufactured from oil. Petroleum products are used not just as gasoline and diesel fuel to power automobiles but also as the basis for hundreds of everyday products including plastics, paints, cosmetics, and detergents. The United States alone uses in excess of 950 billion liters (250 billion gallons) of oil each year, over 350 billion liters of which are imported. When crude oil is transported, some amount of leakage almost always occurs. Tanker accidents spill nearly 400 million liters of crude oil each year, and even larger volumes of oil enter our seas through naturally occurring spills and leaks.

Oil spills have had a tremendous impact on the environment, specifically on large numbers of wildlife (**Figure 17a**). Typically large spills do not have a great effect on human life directly. This is because most large spills occur in open oceans or bays far removed from swimming beaches and water supplies. Humans are more affected by small local spills, such as a gas station's leaking underground tank that may threaten local drinking water supplies.

In 1989, the *Exxon Valdez* oil tanker ran aground on a reef in Prince William Sound off the coast of Alaska, releasing approximately 42 million liters (11 million gallons or about 260,000 barrels) of crude oil and contaminating over 1,000 miles of the Alaskan shoreline. Many experimental approaches for bioremediation were implemented to clean up this spill. Prince William Sound became a field laboratory for trying bioremediation cleanup strategies.

As is done at most sizable oil spills, physical cleaning measures were first used to contain and remove large volumes of oil. These measures included the use of containment booms or skimmers—surface nets, fences, or inflatable devices like buoys that float on the surface—which are fixed in place or towed behind a boat to collect and contain oil (Figure 17b). Then vacuums were used to pump oil from the surface into disposal tanks. Beaches and rocks were washed with fresh water under high pressure to disperse oil. By diluting and dissolving the oil in the sea, the oil was gradually dispersed. One biotechnology cleanup application used citrus-based products to bind crude oil and allow it to be collected on absorbent pads. But after using all these physical approaches to remove the bulk of the oil, millions of gallons of oil still remained attached to sand, rocks, and gravel both at the surface and below the surface of contaminated shorelines. This is when bioremediation went to work.

FIGURE 17 Oil Spills Pose Serious Threats to the Environment (a) Oil spills typically have the greatest impact on wildlife. (b) Containment booms are used initially to control the spread of oil and minimize pollution of the surrounding area.

(a): Jack Smith/AP Images., **(b):** Courtesy of the United States Navy.

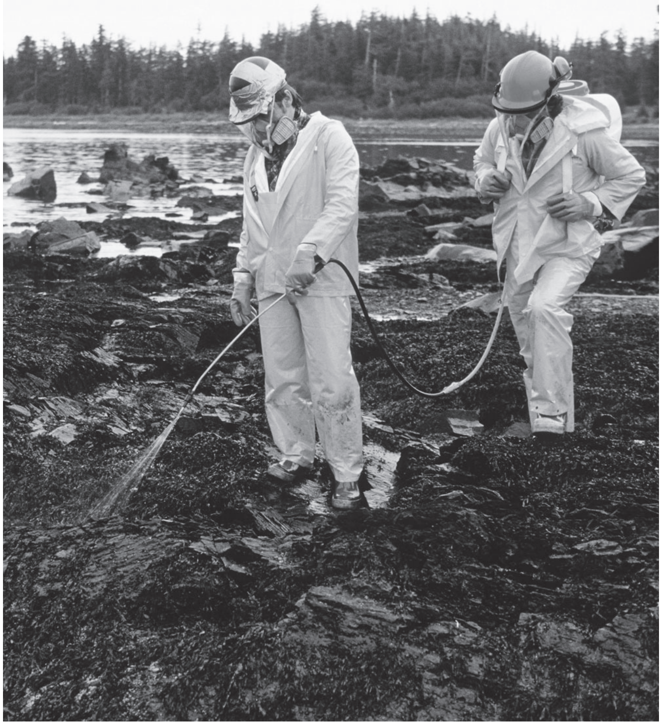

FIGURE 18 Applying Fertilizers to Stimulate Oil-Degrading Microbes Cleanup workers spray nitrogen-rich fertilizers onto an oil-soaked beach in Alaska following the *Exxon Valdez* oil spill. The fertilizers greatly accelerate the growth of natural bacteria that will degrade the oil.

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As the first step in the bioremediation process, nitrogen and phosphorus fertilizers were applied to the shoreline to stimulate oil-degrading bacteria (mostly strains of *Pseudomonas*) that were living in the sand and rocks (**Figure 18**). Indigenous bacteria immediately showed signs that they were degrading the oil. When microbes degrade petroleum products, PAHs are formed and eventually oxidized into carbon chains that can be broken down into carbon dioxide and water. Over time, chemical tests on oil from the shoreline soil showed significant changes in chemical composition, indicating that natural degradation by indigenous bacteria was working. However, oil seeped into sediments and other low-oxygen layers below shoreline rocks, where biodegradation is relatively slow. It may take hundreds of years for the oil spilled by the *Exxon Valdez* to be fully cleared, and some areas of the Alaskan environment may never return to the state in which they were prior to the spill.

Oil Fields of Kuwait

The deserts of Kuwait are literally a living laboratory for studying bioremediation. Ten years after the Gulf War, large areas of Kuwait's desert remain soaked in oil. During the Iraqi occupation of Kuwait from 1990 to 1991, countless oil fields were destroyed and burned, releasing an estimated 950 million liters of oil into the deserts—more than 20 times the amount of oil spilled in the *Exxon Valdez* accident. Kuwaiti scientists studying these spills have found that the spilled oil has severely affected plant and animal life in many contaminated areas. Some plant species have been completely eliminated, and the long-term biological impacts will not be known for many more years.

In contrast to the *Exxon Valdez* spill, bioremediation of desert soils poses a number of different problems. Unlike the spill in Alaska, there are no waves to help disperse and dissolve oil. Dry soil conditions of the desert also tend to harbor fewer strains of oil-metabolizing microbes, and adhesion of oil to sand and rocks slows natural degradation processes. Preliminary studies suggest that novel strains of oil-degrading bacteria are slowly working to break down oil below the surface of the sand.

The Kuwaiti government has developed a $1 billion program to address what may be the world's largest bioremediation project. There are no previously studied sites of this size to use as a model for cleaning up arid desert environments, so bioremediation scientists from around the world are studying Kuwait's deserts in hopes of developing strategies for cleaning up these oil-drenched sands. Information that scientists learn from studying this region will certainly be of value for treating oil spills in other sandy environments.

The *Deepwater Horizon* Oil Spill

On April 20, 2010 British Petroleum's (BP) oil-drilling rig the *Deepwater Horizon* exploded, releasing millions of gallons of crude oil into the Gulf of Mexico about 65 kilometers off the Louisiana coast (**Figure 19a**). Although estimates of oil flow have varied widely, more than 600 million liters (4 million barrels) were released into the Gulf of Mexico before the broken well was capped in mid-July, resulting in the largest marine oil spill in history and predictions that it would cause the world's largest environmental disaster. Yet, given the size of the spill, far less oil than many predicted made it to the coast. Oil that was not removed by cleanup crews was dispersed by wave action and through the use of chemical dispersants that broke up the slick. Controlled burns and surface oil evaporation of volatile materials such as PAHs also contributed to oil cleanup, but bioremediation played a big role accounting for the degradation of an estimated 50% of the oil released.

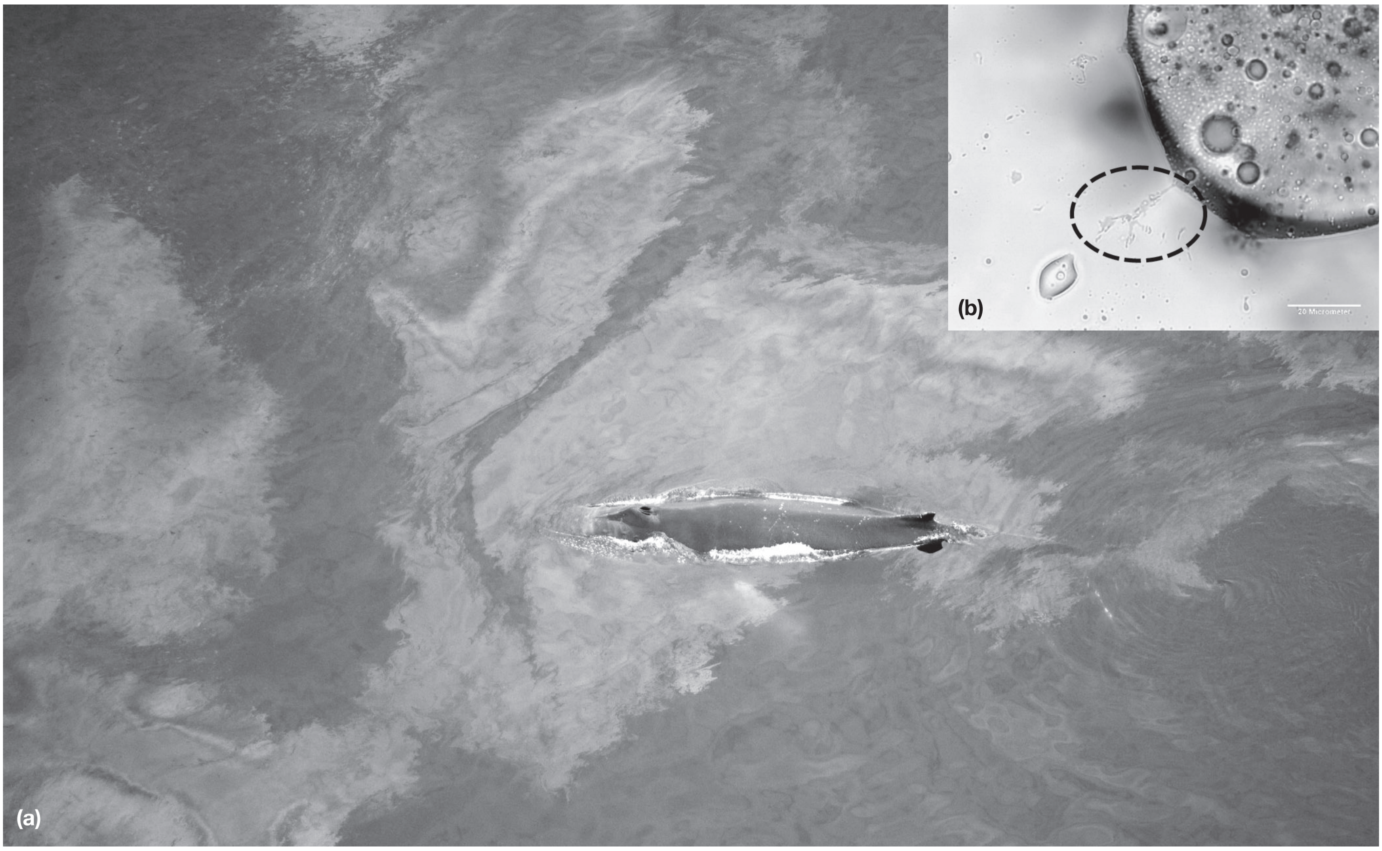

FIGURE 19 Microbes Played a Major Role the Bioremediation of Oil from the *Deepwater Horizon* Oil Spill in the Gulf of Mexico (a) Indigenous microbes that were very abundant in surface and subsurface plumes of oil emanating from the *Deepwater Horizon* spill were essential for bioremediation. Shown here is a whale swimming through a surface plume of oil in the Gulf. (b) An oil droplet magnified 100 times and metabolizing microbes (circled).

(a): Wild Wonders of Europe/Carwardine/bluegreenpictures.com., **(b):** REUTERS/Hoi-Ying Holman Group/Handout.

Studies by oceanographers from the Woods Hole Oceanographic Institute in Massachusetts reported that during the first 5 days of the spill, microbes had not significantly degraded the oil. Over time, an underwater plume of oil that was at least 22 miles long developed. Within weeks, microbes appeared to be flocking to the plume, replicating rapidly, so that bacteria were twice as dense inside the plume compared to outside the plume (Figure 19b). Research on these indigenous microbes in the plume revealed over 1,500 genes encoding proteins designed to degrade hydrocarbons. Clearly hydrocarbon-degrading microbes were highly enriched in the plume, and these microbes were actively breaking down oil. It was also determined that microbes degraded an estimated 200,000 tons of methane that spewed into the Gulf of Mexico during the spill. Ethane and propane were also major hydrocarbons released and these too were degraded by microbes.

The warm waters of the Gulf and components in the plume—such as natural gas, which contains propane and ethane—helped stimulate biodegradation by indigenous microbes. Fertilizers such as iron, nitrogen, and phosphorus were applied at the site to stimulate rates of biodegradation. Gradients in dissolved oxygen levels in the plume also contributed to differences in rates of biodegradation throughout and along the plume. Estimates have suggested that these microbes were reducing oil amounts in the plume by half nearly every 3 days.

Where did they come from? Petroleum-degrading bacteria have existing for eons, thriving on oil that seeps naturally through the seafloor. Each year about 79 million liters of oil leak onto the floor of the Gulf of Mexico through naturally occurring seepage. Within the *Deepwater Horizon* plume, researchers have detected over 900 subfamilies of bacteria, including newly discovered species. It is also likely that chemical dispersants used to break up the stream of oil gushing from the broken well may have created microscopic particles that increased the surface area between oil droplets and water, allowing for greater contact with oil-degrading bacteria. The impact of oil that moved into Louisiana's wetlands and long-term impacts of dispersed oil and chemical dispersants on marine ecosystems in the Gulf region will be closely evaluated in the years to come.

The next section provides a glimpse of how potential applications of bioremediation may be able to solve cleanup problems that have been difficult to treat.

6 Challenges for Bioremediation

Biotechnology is a significant tool in our fight to rehabilitate areas of the environment that have been polluted through accidents, industrial manufacturing, and mismanagement of ecosystems. Bioremediation is a rapidly expanding science. Scientists around the world are carrying out research aimed at developing a greater understanding of the microorganisms involved in biodegradative processes, including the identification of novel genes and proteins involved in these breakdown processes. Researchers are studying microbial genetics to create genetically engineered microbes that may be able to degrade new types of chemicals, and they are developing novel biosensors to detect and monitor pollution.

Recovering Valuable Metals

The recovery of valuable metals such as copper, nickel, boron, and gold is another area of bioremediation that has yet to reach its full potential. Through oxidation reactions, many microbes can convert metal products into insoluble substances, called metal oxides or ores, which will accumulate in bacterial cells or attach to the bacterial cell surface. Some marine bacteria that live in deep-sea hydrothermal vents have also shown potential for precipitating precious metals. Using bacteria as a way to recover hazardous metals as part of industrial manufacturing processes is one potential application, but scientists are interested in finding ways in which these bacteria can be used to recover valuable precious metals. For instance, many manufacturing processes use silver and gold plating techniques; these processes produce waste solutions with suspended particles of silver and gold. Microbes may be used to recover some of these metals from the waste solutions. Similarly, microbes may also be used to harvest gold particles from underground water supplies and cave water found in gold mines. Many bacterial strains that live in such environments are actively being studied for these purposes. Plants may also provide a way to harvest metals from the environment. The wild mustard *Thlaspi goesingense*, native to the Austrian Alps, is known to accumulate metals in storage vacuoles.

Bioremediation of Radioactive Wastes

Another area of active research involves developing bioremediation approaches to remove radioactive materials from the environment. Uranium, plutonium, and other radioactive compounds are found in water from mines where naturally occurring uranium is processed. Radioactive wastes from nuclear power plants also present a disposal problem worldwide. The U.S. Department of Energy (DOE) has identified over 100 sites in 30 states that have been contaminated by weapons production and nuclear reactor development. Radioactive waste sites often have a complex mix of radioactive elements such as plutonium, cesium, and uranium along with mixtures of heavy metals and organic pollutants such as toluene.

Although most radioactive materials kill a majority of microbes, some strains of bacteria have demonstrated a potential for degrading radioactive chemicals. For example, researchers at the University of Massachusetts have discovered that some species of *Geobacter* can reduce soluble uranium in groundwater into insoluble uranium, effectively immobilizing the radioactivity. To date, however, no bacterium has been discovered that can completely metabolize radioactive elements into harmless products.

One bacterial strain in particular, called ***Deinococcus radiodurans*** (**Figure 20**), is especially fascinating. Its name literally means "strange berry that withstands

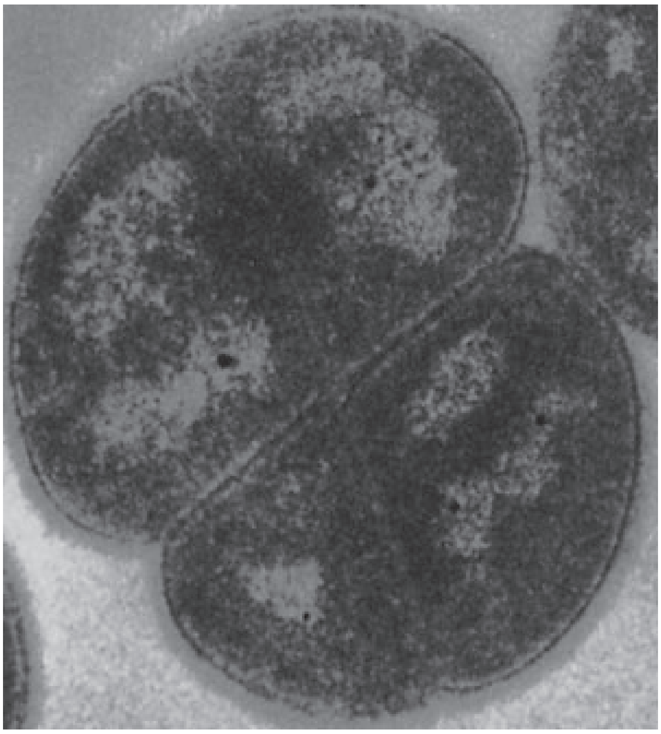

FIGURE 20 ***Deinococcus radiodurans*** These bacteria are highly resistant to damage by radiation.

TEM of D. radiodurans acquired in the laboratory of Michael Daly, Uniformed Services University, Bethesda, MD.

YOU DECIDE

The PCB Dilemma of the Hudson River

Winding through upstate New York, the Hudson River Valley comprises some of the most beautiful country in the Northeast. But serious problems lurk below the glimmering surfaces of the Hudson's blue water. From 1947 to 1977, the General Electric Company released over 1.2 million pounds of toxic chemicals called polychlorinated biphenyls (PCBs) into the river from facilities in Hudson Falls and Fort Edward, New York. PCBs were commonly used in transformer boxes, capacitors, and cooling and insulating fluids of electrical equipment manufactured before 1977. PCBs are no longer used in manufacturing in the United States, and they have been banned in most of the world.

PCBs are very toxic to humans and wildlife because these fat-soluble chemicals gradually accumulate in fatty tissues. Hudson River fish are contaminated with PCBs at concentrations in excess of safe levels. Consumption of fish from most areas of the Hudson is banned or restricted, but some people ignore these publicized restrictions because the water looks so clear and the fish do not *look* polluted. PCBs present serious health threats to humans, and prolonged exposure to PCBs is known to cause cancer, reproductive problems, and other medical conditions affecting the immune system and thyroid gland. Children are particularly susceptible to the health effects of PCBs.

High concentrations of these chemicals still lurk in the Hudson River and other environments. In the Hudson River, most PCBs are located in the sediment at the bottom of the river. To get rid of these persistent chemicals, environmental groups and the EPA have proposed dredging the 175-mile-long riverbed to remove over 2.5 million cubic yards of sediment, which would remove more than 100,000 pounds of PCBs. Critics of the dredging plan suggest that such operations would only release more PCBs into the water by stirring up the sediment. Instead of dredging, many believe that leaving the sediment in place and letting natural current flows disperse the chemicals, combined with bioremediation through bacterial degradation of PCBs, is the best way to reduce the load of PCBs in the long run.

PCB-degrading anaerobes have been detected in Hudson River sediments. Some anaerobic bacteria are involved in the first step of breaking down PCBs by cleaving off chlorine and hydrogen groups. Aerobic bacteria in the water can further modify PCBs, and others can then convert them into water, carbon dioxide, and chloride. Some predictions suggest that even after dredging, PCB levels in fish will not drop to levels acceptable for human consumption until after 2070. Even if dredging is a good plan, where will the dredged sediments go? How will these chemicals be cleaned up? Some people believe that placing PCB-laden sediments in a sealed landfill, a completely anaerobic environment, will slow down the degradative processes. Should these chemicals be left in the mud to be broken down slowly over time through natural bioremediation, or should humans intervene in an effort to speed up nature's cleanup effort? What are the pros and cons of taking action or doing nothing?

You decide.

radiation." Named the world's toughest bacterium by *The Guinness Book of World Records*, *D. radiodurans* was first identified and isolated about 50 years ago from a can of ground beef that had spoiled, even though it had been sterilized with radiation. Subsequently, scientists discovered that *D. radiodurans* can endure doses of radiation over 3,000 times higher than other organisms can, including humans. High doses of radiation create double-stranded breaks in DNA structure and cause mutations, yet *D. radiodurans* shows incredible resistance to the effects of radiation. Although its resistance mechanisms are not entirely known, this microbe clearly possesses elaborate systems for folding its genome to minimize damage from radiation, and it also uses novel DNA repair mechanisms to replace damaged copies of its genome. Recent completion of the *D. radiodurans* genome map is expected to provide valuable insight into its unique DNA repair genes.

The DOE is very interested in using *D. radiodurans* and another bacterium, *Desulfovibrio desulfuricans,* for bioremediation of radioactive sites. Recently researchers at the DOE and the University of Minnesota created a recombinant strain of this microbe by joining a *D. radiodurans* gene promoter sequence to the gene encoding the enzyme toluene dioxygenase which is involved in toluene metabolism. This strain demonstrated the ability to degrade toluene in a high-radiation environment. In an effort to immobilize radioactive elements, scientists