

Healthy Environments for Healthy People: Bioremediation Today and Tomorrow

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Increases in environmental contamination lead to a progressive deterioration of environmental quality. This condition challenges our global society to find effective measures of remediation to reverse the negative conditions that severely threaten human and environmental health. We discuss the progress being made toward this goal through application of bioremediation techniques. Bioremediation generally utilizes microbes (bacteria, fungi, yeast, and algae), although higher plants are used in some applications. New bioremediation approaches are emerging based on advances in molecular biology and process engineering. Bioremediation continues to be the favored approach for processing biological wastes and avoiding microbial pathogenesis. Bioremediation may also play an increasing role in concentrating metals and radioactive materials to avoid toxicity or to recover metals for reuse. Microbes can biodegrade organic chemicals; purposeful enhancement of this natural process can aid in pollutant degradation and waste-site cleanup operations. Recently developed rapid-screening assays can identify organisms capable of degrading specific wastes and new gene-probe methods can ascertain their abundance at specific sites. New tools and techniques for use of bioremediation *in situ*, in biofilters, and in bioreactors are contributing to the rapid growth of this field. Bioremediation has already proven itself to be a cost-effective and beneficial addition to chemical and physical methods of managing wastes and environmental pollutants. We anticipate that it will play an increasingly important role as a result of new and emerging techniques and processes. — *Environmental Health Perspectives* 105(Suppl 1):5–20 (1997)

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Environmental Contamination and Bioremediation

The publication of Rachel Carson's *Silent Spring* in 1962 (1) spoke to the American public about the direct link between the health of the ecological environment and the health of humanity. Since that time, all facets of American society have stepped up their efforts to prevent environmental degradation. Congress passed the Clean Air Act and the Clean Water Act and established

the U.S. Environmental Protection Agency (U.S. EPA). Study results of the extra- and intramural research programs of the National Institute of Environmental Health Sciences have contributed to the increasing public awareness that human diseases often have preventable environmental components. Pollution prevention and environmental remediation are interwoven into all strategies proposed for sustaining human and environmental health. Remediation based on pollutant metabolism or absorption

by normal, selected, and/or genetically engineered microbes is emerging as a distinctive and promising approach to cleaning up polluted environments. Harnessing microbial processes for good, rather than experiencing their harmful attributes as propagators of disease, is the goal of bioremediation. In the following review we describe current and emerging measures of remediation based on biologically active cells and organisms and focus on microbial processes. Microbes play an essential role in nature's cycles and they are the primary stimulant in bioremediation of contaminated environments (2–7). In the natural cycles for the transformation of mercury, for example, bacteria are important for most of the reactions illustrated in Figure 1.

Most elements exist in a variety of forms that differ in their availability and toxicity to humans and to other forms of life. To meet the challenges presented by environmental pollution, the goal of bioremediation (together with prevention and chemical and physical approaches to remediation) is to reduce the amount and availability of hazardous chemical compounds and convert them to useful or at least innocuous products.

Bioremediation systems generally utilize microbes (bacteria, fungi, yeast, and algae) or microbial products for the degradation or concentration of waste, although in some cases higher plants are also being developed for this purpose (8–11). Novel metabolic opportunities are introduced as a result of species selection. For example, enzymes found in the fungus *Phanerochaete chrysosporium* (white rot fungus) effectively degrade some wastes that prove resistant to most bacterial action (e.g., DDT and 2,4,5-trichlorophenoxyacetic acid).

The need for a biological approach to improve environmental conditions directly relates to the increasing size of the human population on a planet of finite dimensions. The estimated population of the earth in 1996 is 6 billion people, but by the year 2100 the number is expected to almost double (12). Whereas the number of population doublings that might be sustained by advances in technology (without bringing unbearable pain and suffering) may be argued, no one believes that such an increase can go on indefinitely, and there are already great inequities in degree of pollution-related suffering among populations (13,14). As populations grow in size, increases in a variety of adverse human

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Abbreviations used: GE, General Electric; PCBs, polychlorinated biphenyls; RCRA, Resource Conservation and Recovery Act; STP, sewage treatment plants; TCE, trichloroethylene; U.S. EPA, U.S. Environmental Protection Agency; VOCs, volatile organic compounds.

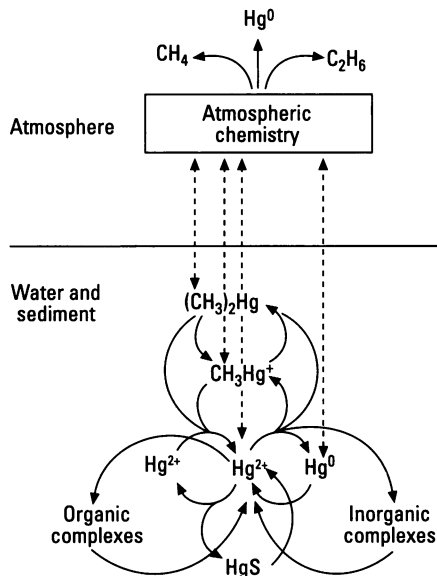


Figure 1. The transformation, cycling, and movement of mercury in the environment. Mercury transformations are made primarily by the action of bacteria. Physical conditions, such as temperature and pH, also have major role-determining equilibrium values in mercury transformations, and thus availability to man. Anthropogenic input of mercury into the environment also affects the equilibrium. Unlike lead, for which there are monitoring data to show appreciably decreased concentrations in the atmosphere in recent years, the concentration of mercury seems to be increasing.

health and ecological effects (and associated costs such as health care expenses) are also expected.

The U.S. EPA's Toxic Substances Control Act Chemical Inventory includes over 72,000 chemicals, with approximately 2300 new chemicals submitted to the U.S. EPA every year (15,16). Along with population increases, the number of different chemicals and the total amount of chemicals produced are also bound to increase in the future. In 1990, the total release of toxicants into the environment by U.S. manufacturers was approximately 4.8 billion pounds (17). In addition, large quantities of a number of toxic products are released into the environment by end users in more or less unaltered form. These products include those designed for household use as well as industrial materials such as fuels, detergents, fertilizers, dielectric fluids, preservatives, flavorings, flame retardants, heat transfer fluids, lubricants, protective coatings, propellants, pesticides, refrigerants, and many other chemicals. Such materials or their breakdown products often accumulate in soil and aquifers near landfills and dumps, in surface lakes and

streams, and in sediment. These pollutants are present not only in concentrated waste sites but are widely distributed throughout the environment, although in many cases at levels too low to trigger regulatory action. The kinds and amounts of these chemicals are also likely to increase as populations of humans swell.

Bioremediation is not new to the human race, although new approaches that stem from advances in molecular biology and process engineering are emerging. An important, long-standing, and increasingly problematic bioremediation area is processing biological nitrogen waste (feces and urine) produced by humans and the animals that humans depend on for food. As human population size, industrial production, and chemical use have increased, so have populations of farm animals. In North Carolina alone, approximately 27,000,000 tons of fresh manure containing 205,000 tons of nitrogen, 138,000 tons of phosphorus, and 133,000 tons of potassium were generated by food animals in 1993 (18). Much of this waste ends up in river waters and estuaries, where it causes enormous problems, with secondary contributions to air and groundwater pollution (19–23). North Carolina's top health official, J.B. Howes, Secretary of the North Carolina State Department of Health, Environment, and Natural Resources, has declared that bad water quality, caused primarily by animal and human nitrogen waste, is North Carolina's number one environmental health problem (J.B. Howes, personal communication). The poor water quality dead zone in the Gulf of Mexico off the Louisiana coast reportedly covered more than 6000 mi² in 1995 (24). It is no wonder; worldwide, the effects of poor water quality are second only to malnutrition in the total disease burden and cause of death of human beings (25).

Because of the importance of clean water to human health, sewage treatment plants (STPs) constitute the largest and most important bioremediation enterprise in the world. There are approximately 16,000 municipal STPs in the United States, which process about 40 billion m³ of raw sewage per year (5). The major components of raw sewage are suspended solids, organic matter, nitrogen, phosphorus, pathogenic microorganisms, and chemicals (e.g., pesticides and heavy metals), and even the most rudimentary STPs make some reductions in most of these factors. A variety of methods is used for sewage treatment. Generally, primary

treatment consists of a screening device to remove a variety of large trash and debris (usually hauled away to landfills), a settling tank where coarse grit and sand particles are removed, and a primary clarifier (essentially a large tank from which floating solids and settled sludge are removed after the sewage has resided in the tank for a brief period of usually a few hours). The limited time in the primary clarifier means that microorganisms living in the tank do not have the opportunity to consume a large amount of the nutrient material contained in the sewage. The floating solids and the sludge are then pumped to an anaerobic digester. The liquid effluent is disinfected, usually with chlorine, before its release into the environment. Alternatively, additional processes, referred to as secondary- and tertiary-level sewage treatments, may be applied to further reduce the levels of nutrients, pathogens, and chemicals. Of course, these additional treatments cost money—usually from taxpayer dollars.

The anaerobic digester contains microorganisms adapted to grow and multiply in the absence of oxygen at elevated temperatures. In this process nutrients are converted primarily to microbial biomass, methane, and carbon dioxide and thus are consumed. The liberated methane is used to heat the digester. The sludge coming out of the anaerobic digester has considerably reduced objectionable qualities (less odor as well as reduced numbers of pathogens) and is typically transported to a landfill or applied to the land as fertilizer.

Secondary sewage treatment consists of two main types: trickling filters and activated sludge. Trickling filters are cylindrical tanks containing loosely packed rocks, which range in size from 2–10 cm. Effluent enters through the top; air is introduced from the bottom. Distributed throughout the column is a variety of organisms that are attached to the surfaces of the rocks and in the intervening spaces. Bacteria and fungi are the first to consume the organic constituents, and in turn the bacteria and fungi are consumed by higher trophic level organisms, including protozoa, rotifers, nematodes, worms, and insects. Activated sludge systems consist of a series of tanks. Effluent is introduced at one end, and it exits at the other. In between, the sewage is mixed and aerated vigorously. Bacteria are the main decomposing organisms in the activated sludge system, but protozoans, rotifers, and nematodes are also present. All the various life forms tend to occur together in flocculant masses.

Both activated sludge and trickling filter secondary STP systems can be effective, but there are advantages and disadvantages to each. Trickling filters seem to be more tolerant of industrial chemicals, perhaps because of greater species/metabolic diversity. However, trickling filters require more space, cost more to construct, and tend to create more of an odor problem. Activated sludge systems tend to achieve greater reductions in organic nutrients and suspended solids.

Regardless of which secondary process is used, without further (i.e., tertiary) treatment, large amounts of nitrogen and phosphorus remain in secondary STP effluent (26). These inorganic nutrients in turn encourage algal and phytoplanktonic growth in receiving waters. Ultimately, these organisms die and decompose, which consumes oxygen and thereby promotes hypoxic and anoxic conditions. Fish kills resulting from oxygen deprivation are notable consequences; in extreme cases, millions of fish are killed (23,27,28). The technology to remove both nitrogen and phosphorus (and as a result, counteract these effects) has been available for some time (29). Inorganic phosphorus can be precipitated from solution by the addition of calcium (as lime, CaO), aluminum (as alum, aluminum sulfate), or a variety of other relatively inexpensive chemicals. Nitrogen can be removed both chemically and biologically. Most of the nitrogen in secondary sewage effluent occurs as ammonium ion (NH_4^+). The process of ammonia stripping involves the conversion of NH_4^+ to ammonia gas (NH_3) by raising the pH along with vigorous agitation. However, the liberated ammonia gas then becomes a potential atmospheric pollutant. Biological conversion of nitrogen gas (N_2) by denitrifying bacteria is an alternative approach, although there are other approaches as well, e.g., break point chlorination, reverse osmosis, and distillation (30,31). In spite of the available technology, implementation has been limited, and eutrophication, caused in part by the effluent of STPs, still commonly occurs in many coastal regions throughout the world.

The discharge of STP effluent on land rather than in water has been tried many times, often with at least initial success (32–35). The potential advantages of land deposition are that groundwater resources can be recharged and that valuable nutrients become available to assist with crop growth and other vegetation. Disadvantages include possible groundwater contamination

with nitrates [NO_3^- , associated with methemoglobinemia in infants, cancer, and birth defects (36,37)] and other toxic, possibly carcinogenic, chemicals, including biocides (38). Other disadvantages are the increased risk of exposure to disease pathogens and the gradual accumulation of heavy metals in soils such that the growth of crops can eventually become inhibited (39). In spite of these problems, land application of STP effluent has been remarkably useful in many cases, e.g., the reclamation of strip-mined soil (40,41).

It is estimated that more than half of the rainwater that falls on the United States is converted to wastewater by people, cities, and industry (42). Although there are many less-than-ideal systems, bioremediation carried out in STPs does a reasonable overall job of cleaning up this huge amount of waste. Agricultural operations, on the other hand, sometimes do not tend to their animal wastes. Sixty percent of water quality impairment is attributed to silt and fertilizer runoff (42).

Septic tanks are another large and imperfect bioremedial system that contributes nitrogen and other waste to the impairment of water quality, particularly to groundwater. Patrick et al. (43) estimated that almost one-third of the U.S. population still relies on septic tanks and that they handle roughly 15 billion liters per day of sewage. U.S. EPA studies (44) indicate that about one-third of all septic tanks operate improperly; as a result septic tanks are the primary source of groundwater contamination in many parts of the country. This contamination leads to nitrate, chemicals, and pathogens in the well water that some people drink.

The risk of pathogenic disease associated with nitrogen waste should not be underestimated. Human waste, in the form of sewage treatment plant sludge, for instance, contains significant amounts of bacterial, viral, and other pathogens even after stabilization and treatment (45). Some of these pathogens can remain viable for long periods of time and contaminate groundwater located below the sludge deposit locations. Huge areas deep beneath the sea still contain viable pathogens as a result of offshore ocean disposal of sewage waste (46). Pathogens are also found in animal waste, and the presence of nitrogen together with microbial contamination may exacerbate the expression of disease.

Improved bioremediation of biological wastes is envisioned as a necessary first step in breaking the chain of events associated

with microbial pathogenesis. In England, the recent outbreak of bovine spongiform encephalopathy (Mad Cow Disease) that is believed to be associated with Creutzfeldt–Jakob disease in humans has increased concern over disease transmission from food animals to humans (47,48). In fact, a great many microbial diseases (zoonotic diseases) can and often do cross over to affect humans. Diseases that can pass to humans from swine, for example, include:

- bacterial infections, such as anthrax (*Bacillus anthracis*), brucellosis (*Brucellosis suis*), ampylobacteriosis (*Campylobacter jejuni*), erysipeloid (*Erysipelothrix rhusiopathiae*)
- viral infections, such as encephalomyocarditis (*Cardiovirus*), influenza (*Influenzavirus*), Japanese B encephalitis [*Flavivirus* (gp A)], and vesicular stomatitis (*Vesiculovirus*)
- nematode infections, such as ascariasis (*Ascaris suum*) and trichinosis (*Trichinella* spp.)
- protozoan infections, such as balantidiasis (*Balantidium coli*), toxoplasmosis (*Toxoplasma gondii*), amoebic dysentery/amebiasis (*Entamoeba polecki*) and sarcocystosis (*Sarcocystis suihominis*)
- spirochetal infections, such as leptospirosis (*Leptospira interrogans*) (49,50).

Although the advent and continued development of antibiotics have kept infectious disease in developed countries under control for many years, there is growing evidence that this may not be effective indefinitely, as increasingly virulent and antibiotic-resistant strains continue to evolve (51–53).

Two additional classes of pollutants also continue to be of enormous practical and economic importance and as a result merit special mention in any discussion of bioremediation. The first is the inorganic pollutant category of heavy metals, such as lead, mercury, and cadmium. These natural elements, found in the Earth's crust, are utilized in many industrial processes and products, a use which has resulted in their release in higher concentrations and in more accessible form than is typical in natural systems. Incorporation of heavy metals into inorganic and organometallic complexes often alters their biological activity; such changes are just as likely to increase toxicity, due to increased bioavailability, as they are to decrease toxicity. Furthermore, depending on conditions of pH, increased temperature, etc., natural cycles may intervene to convert or mobilize relatively benign inorganic species to more

toxic organic complexes, e.g., conversion of elemental mercury to methylmercury (Figure 1).

A second class of pollutants is radioactive waste materials. Radioactive compounds, although they may be biologically converted from one form to another,

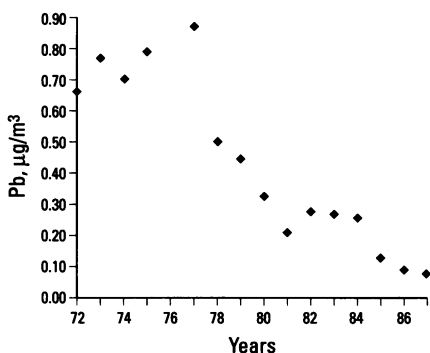


Figure 2. Average yearly lead (Pb) concentrations in ambient air in North Carolina. The decline represents the results of removing lead from gasoline. Currently, the most important sources of lead in air are thought to result from the sandblasting of bridges and water tanks, many of which are still coated with lead-based paint. Children playing in lead contaminated areas are particularly at risk.

ultimately yield other radioactive metabolites, which can be as hazardous or more so than the parent compound. However, certain radioactive substances, such as uranium, can be immobilized, concentrated, and removed from the environment with the aid of suitably adapted microorganisms, e.g., *Citrobacter* sp. (54) or appropriate biomass (55–58).

Unlike organic pollutants, the toxicity of metals is inherent in their atomic structure, and they cannot be further transmuted/mineralized to a totally innocuous form. Their oxidation state, solubility, and association with other inorganic and organic molecules can vary, however; microbes as well as higher organisms may play a bioremediative role by concentrating metals so that they are less available and less dangerous.

Because of the proven health danger of heavy metals, federal and state governments routinely monitor the environment for their presence. In cases where control strategies have been implemented, there have been significant decreases in environmental metal levels. Figure 2 shows recent levels of atmospheric lead in North Carolina (59). The sharp decline is largely the result of

removing lead from gasoline. For other heavy metals, the results are less encouraging. Mercury, for instance, seems to be increasing in the environment in spite of environmental control measures (60–62).

There has been great interest in mercury contamination since the 1950s, when hundreds of people in Japan became seriously ill and many died from eating seafood that was contaminated with methylmercury. North Carolina is one of many states in the United States that now routinely monitor freshwater fish for mercury levels. Many fresh- and saltwater fish have mercury levels greater than the alert levels designated by the U.S. Food and Drug Administration and the World Health Organization. Fish in North Carolina with reported mercury concentrations greater than 1 ppm (range: 1–6.9 ppm) in the edible (filet) portion include the following:

- *Ictalurus punctatus* (channel catfish)
- *Ictalurus (Ameriurus) catus* (white catfish)
- *Ictalurus (Ameriurus) natalis* (yellow bullhead)
- *Ictalurus (Ameriurus) nebulosus* (brown bullhead)
- *Ictalurus (Ameriurus) platycephalus* (flat bullhead)

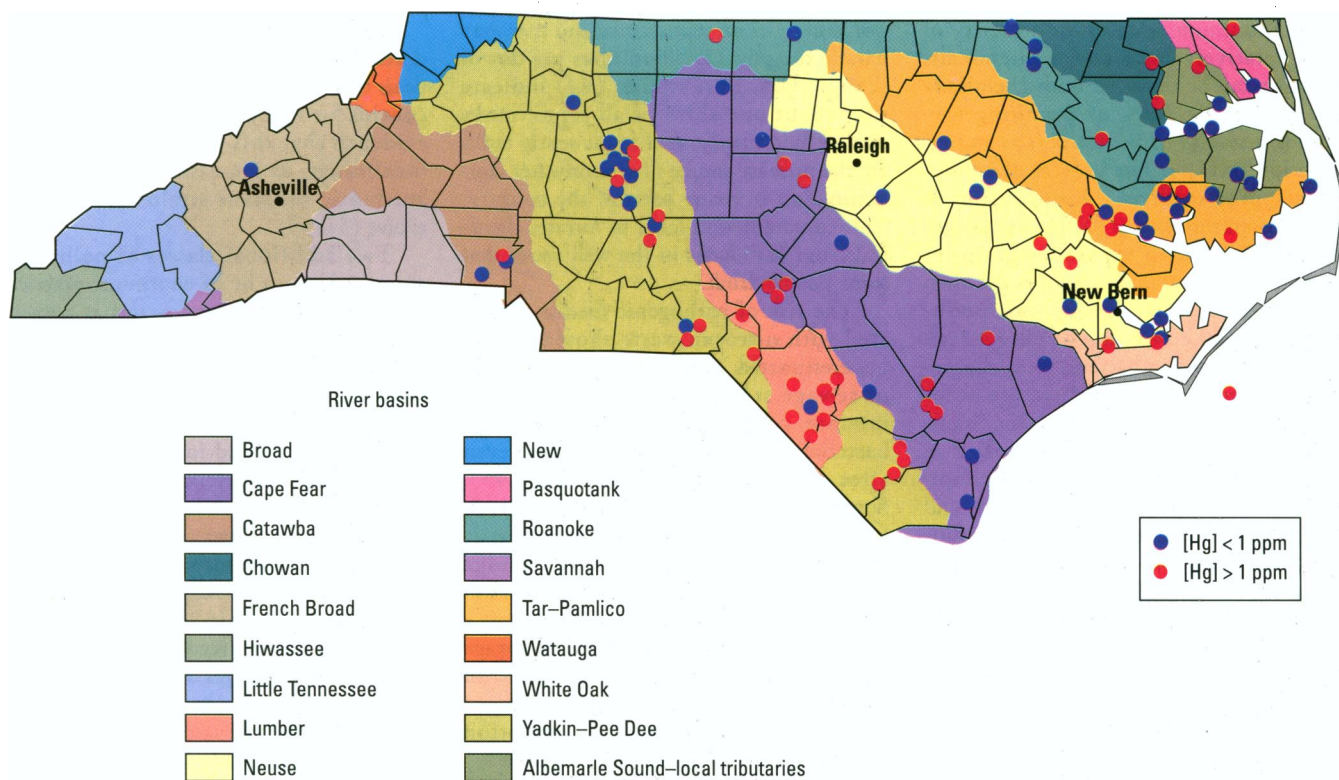


Figure 3. Map of North Carolina showing sampling sites from which fish were collected and analyzed for the presence of mercury. The state's watersheds are indicated by color. Sites where some fish contained greater than 1 ppm mercury are indicated, as are sites where some fish contained less than 1 ppm mercury.

- *Lepisosteus osseus* (longnose gar)
- *Carassius auratus* (goldfish)
- *Micropterus salmoides* (largemouth bass)
- *Cyprinus carpio* (common carp)
- *Pomoxis nigromaculatus* (black crappie)
- *Lepomis gulosus* (warmouth)
- *Lepomis auritus* (redbreast sunfish)
- *Lepomis gibbosus* (pumpkinseed sunfish)
- *Lepomis macrochirus* (bluegill)
- *Esox niger* (chain pickerel)
- *Amia calva* (bowfin)
- *Carcharhinus obscurus* (dusky shark)
- *Carcharhinus plumbus* (sandbar shark)
- *Carcharhinus limbatus* (black-tipped shark)
- *Odentaspis taurus* (sandtiger shark)
- *Sphyrna lewini* (scalloped hammerhead shark)
- *Galeocerdo curvieri* (tiger shark)
- *Makaira nigricans* (blue marlin)

Figure 3 is a map of North Carolina showing sampling sites where fish with 1 ppm or more mercury have been found. These data were collected by the North Carolina State Department of Health, Environment and Natural Resources, beginning in 1980. They are available under the STORET database (U.S. EPA Water Data Storage and Retrieval Data Base, U.S. EPA, Research Triangle Park, NC) maintained by the U.S. EPA but are otherwise previously unpublished. The data show that there are no locations in North Carolina that can be considered free of a potential mercury problem. Although no adverse human effects attributable to mercury poisoning have been reported in North Carolina, some areas have posted signs to warn people not to consume large amounts of fish. This problem occurs in other parts of the country, e.g., Arkansas, South Carolina, Georgia, Florida, Wisconsin, and the Great Lakes region (63–68). Declines in populations of fish-eating wildlife, such as loons, mink, otter, and wild cats are possibly attributable, in part, to environmental chemicals such as mercury. In humans the subtle neurological effects of chronic low-level mercury poisoning might not be obvious.

Many plants and bacteria have evolved various means of extracting essential nutrients, including metals, from their environment. In the course of prospecting for minerals, unusually tolerant species have been observed in the vicinity of metal-rich deposits. In some cases, these tolerant organisms concentrate metals several thousandfold over ambient concentrations. Some examples are listed in Table 1 and in Zajic (69), Antonovics and Bradshaw (70),

and Baker and Brooks (71). As these and other authors, e.g., Shann (72), point out, such organisms may provide the opportunity to return waste material to useful products rather than merely transform them to innocuous substances. However, a practical phytoremediation technology remains to be developed, although progress has been made with transgenic *Arabidopsis thaliana* expressing *merApe9* (73). Grown on medium containing HgCl_2 , at concentrations of 25 to 100 M (5–20 ppm), these transgenic *merApe9* seedlings evolved

considerable amounts of Hg^0 relative to control plants. However, the transformation of ionic mercury to the metallic elemental form, which then volatilizes to become an air pollutant, is a less than ideal remedial solution.

The recovery of metals by microbes or microbial products is more advanced. For example, many microorganisms secrete high-affinity, metal-binding compounds called siderophores. The siderophores bind specific chemical forms of metals, and the metal-siderophore complex then absorbs

Table 1. Examples of plants that hyperaccumulate metal.

Genus and species	Family	Metal concentration, $\mu\text{g/g}^{a,b}$
Cobalt		
<i>Aeollanthus bififormifolius</i>	Lamiaceae	2820
<i>Alectra sessiliflora</i>	Scrophulariaceae	2782
<i>Anisopappus davyi</i>	Asteraceae	2650
<i>Crotalaria cobalticola</i>	Fabaceae	3010
<i>Cyanotis longifolia</i>	Commelinaceae	4200
<i>Haumaniastrum homblei</i>	Lamiaceae	2633
<i>Haumaniastrum robertii</i>	Lamiaceae	10200
Copper		
<i>Aeollanthus bififormifolius</i>	Lamiaceae	3920
<i>Buchnera henriquesii</i>	Scrophulariaceae	3520
<i>Bulbostylis mucronata</i>	Cyperaceae	7783
<i>Eragrostis boehmii</i>	Poaceae	2800
<i>Gutenbergia cupricola</i>	Asteraceae	5095
<i>Haumaniastrum katangense</i>	Lamiaceae	8356
<i>Ipomoea alpina</i>	Convolvulaceae	12300
<i>Lindernia perennis</i>	Scrophulariaceae	9322
<i>Pandiaka metallorum</i>	Amaranthaceae	6260
<i>Vigna dolomitica</i>	Fabaceae	3000
Nickel		
<i>Alyssum argenteum</i>	Brassicaceae	29400
<i>Alyssum masmenaeum</i>	Brassicaceae	24300
<i>Bornmueller baldacci tymphaea</i>	Brassicaceae	31200
<i>Geissois intermedia</i>	Cunoniaceae	22900
<i>Geissois pruinosa</i>	Cunoniaceae	34000
<i>Hybanthus austrocaledonicus</i>	Violaceae	25500
<i>Peltaria emarginata</i>	Brassicaceae	34400
<i>Phyllanthus serpininus</i>	Euphorbiaceae	38100
<i>Psychotria douarrei</i>	Rubiaceae	47500
<i>Thlaspi alpinum sylvium</i>	Brassicaceae	31000
Lead		
<i>Armeria maritima halleri</i>	Plumbaginaceae	1600
<i>Thlaspi alpestre</i>	Brassicaceae	2740
<i>Thlaspi rotundifolium cepaeifolium</i>	Brassicaceae	8200
Manganese		
<i>Macadamia neurophylla</i>	Proteaceae	51800
<i>Maytenus bureauvianus</i>	Celastraceae	33800
<i>Maytenus sebertiana</i>	Celastraceae	22500
Zinc		
<i>Thlaspi alpestre</i>	Brassicaceae	25000
<i>Thlaspi calaminare</i>	Brassicaceae	39600
<i>Thlaspi caerulescens</i>	Brassicaceae	27300
<i>Thlaspi tatraense</i>	Brassicaceae	27000
Mercury		
<i>Jungermannia vulcanicola</i>	(Liverworts)	13000 ^c
<i>Scapania undulata</i>	(Liverworts)	4700 ^d

^aDry weight basis. ^bData from Baker and Brooks (71) except where noted. ^cData from Satake and Miyasaka (139).

^dData from Sameck-Cymerman and Kempers (140).

back into the organism for utilization. Siderophores are an evolutionary adaptation used particularly for supplying iron to cells (74). Iron is an essential element that is often not bioavailable in sufficient amounts in the environment. A bioremediation technology in development will deal with metal pollution and make use of native and chemically modified siderophores attached to inert support media. This approach has been used with some success in the removal of plutonium, uranium, and thorium from radioactive waste (75). Premuzic et al. (76) discovered that uranium and thorium induce siderophore production in *Pseudomonas aeruginosa*. Enterochelin (Figure 4) is an example of a siderophore (77).

Another bioremediative mechanism to deal with metal contamination is bioadsorption. Many bacteria and algae have a cell wall or envelope that is capable of passively adsorbing rather high levels of dissolved metals, usually via a charge-mediated attraction. Cell-wall constituents that make this possible include carbohydrate polymers, peptidoglycans, and melanins (78). Other bacteria absorb and utilize mineral complexes and have evolved special ways of packaging and handling the residual metabolites containing heavy metal as a protection against metal toxicity. Often this process culminates in secretion of heavy-metal precipitates or insoluble complexes. Manipulation of nutrient levels in culture and environmental conditions can stimulate such activity and lead to remarkable concentration efficiencies for metals

such as arsenic, cadmium, chromium, cobalt, copper, nickel, mercury, tellurium, and zinc (79). Appanna et al. (80) recently described a *Pseudomonas fluorescens* model with potential application in the management of cesium from nuclear industries. Practicable bioremediation technologies exploiting these abilities have yet to be perfected, but the potential for metal-concentrating bioreactors has been recognized and is the object of current research.

Perhaps the best-known example of microbial metal metabolism is the mining microbe *Thiobacillus ferrooxidans* (81,82). This bacterium and other related species derive energy by breaking down metallic sulfides. Many heavy-metal ores, such as copper, gold, cadmium, lead, mercury, uranium, and zinc, often occur as complexes with sulfur. Microbial metabolism of the sulfide releases the free metal; sulfuric acid is produced as a by-product, which contributes to further metal leaching. Technologies utilizing *T. ferrooxidans* as an inexpensive, nonpolluting alternative to high-temperature ore smelting have been in limited use since the late 1970s, and development of larger scale applications continues (83). Proposed bioremediation uses of sulfur-oxidizing bacteria include removal of sulfur from coal and fuel oil and use in biofilters to scrub sulfur dioxide (SO₂) from various industrial emissions. Although heavy metals are not actually accumulated by such bacteria, there is potential for bioreactor treatment of metal-contaminated solid wastes, thus resulting in extraction of valuable metals for reuse, especially when sulfides are also present in high concentration. Bioreactor designs that utilize sulfide-oxidizing microbes to release heavy metals from contaminated soils and sludges are now being explored, with promising early results (84). Other bacteria catalyze the reverse reaction: anaerobic reduction of sulfates to sulfides. Sulfides produced in this manner readily complex with heavy metals and precipitate. Many commercial ore deposits are believed to have been produced by biogenesis (69). Sulfate-reducing bacteria may prove to be valuable for precipitation of dissolved metals in waste slurries in anaerobic bioreactor technology.

Adaptable Microbial Metabolism

Microbes can be encouraged to biodegrade almost any organic chemical. Environmental chemists and microbial ecologists have extensively characterized the natural

biodegradation pathways of a number of pollutant classes; recent reviews have been published for many, including polycyclic aromatic hydrocarbons (85), polychlorinated biphenyls (PCBs) (86,87), and pesticides (88,89). Rapid screening assays are being developed by researchers to identify organisms capable of degrading specific wastes (90). Molecular probes make it possible to test a small, mixed microbial population for specific degradative enzyme genes (91). Gene probing can also give an indication of the natural abundance of organisms with the potential to degrade specific pollutants at a given site.

Some organisms have the capacity to utilize an environmental contaminant as a food source and thus grow and multiply more prolifically in areas where the contaminant is present. Mutation and selection may also result in the evolution of microbial strains adapted to utilize environmental contaminants. Consequently, organisms with the highly developed capability to metabolize specific contaminants can be found in contaminated sites. These organisms can be isolated from the microbiological population and cultured for inoculation of other sites or for use in bioreactors. This approach has successfully isolated bacterial strains capable of metabolizing marine crude-oil spills (92), jet fuel (93), and organohalogenes in groundwater aquifers (94,95). Organisms isolated in this way have been used in a number of land treatment and bioreactor systems.

In contrast to the selection of genetically able microorganisms found in a natural environment, a number of researchers focus on developing genetically engineered strains of microorganisms where deliberate manipulation of DNA sequences yields capabilities that the organisms did not previously possess. Although genetically engineered microbes hold considerable promise, their use in bioremediation applications will require further study to clarify issues of safety and containment (96).

Bioremediation Now?

Environmental contamination results in increased health-care costs because many human health problems have an environmental component and, according to some experts, the health-care system is already failing and in crisis (97). In addition, the loss of biodiversity resulting from degradation of environmental health threatens multibillion dollar global industries, such as agriculture, biotechnology, pharmaceuticals, and nature tourism (98). Recent

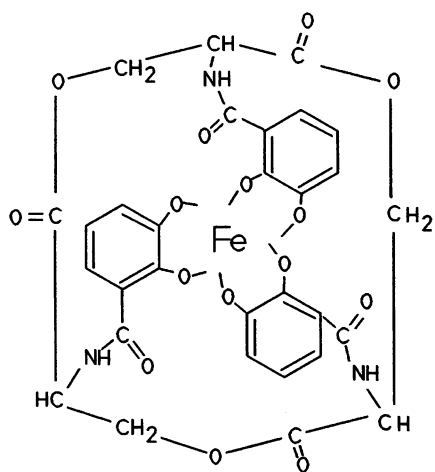


Figure 4. Enterochelin, shown binding an atom of iron, is a siderophore produced by *Klebsiella pneumoniae*. Microorganisms secrete and reabsorb siderophores as means of obtaining iron.

emphasis on sustainable development has focused global attention on the need to adopt environmentally friendly industrial approaches, not only to maintain the Earth's life-support systems, but to ensure the future of natural resources and the economies that they support (98).

It is proving to be not only environmentally advantageous but also politically and economically sound to clean up polluted air and water systems. Enactment of federal legislation, such as the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 and the Superfund Amendments and Reauthorization Act of 1986, has made responsible parties liable for the costs of waste-site cleanup—costs that can be enormous. In addition to actual Superfund remediation, efforts to avoid liability for EPA-administered cleanups under the terms of these statutes have led to large-scale remediation efforts by private industrial concerns in the United States. Therefore, an obvious motivation to remediate is legal compliance. The immediate stimulus may be direct regulatory agency action or cleanup requirements related to real estate transactions. In many states property titles cannot be legally transferred unless the liability for any contamination discovered and cleanup required is assigned. The increased need for remediation of diverse classes of waste and waste sites has created a demand for improved remediation techniques and for techniques that are applicable to a wider variety of cases. As an emerging technology, bioremediation is poised for rapid development.

A second motivation for bioremediation is the dramatic increase in the cost of traditional waste treatment methods, especially bulk disposal by incineration or landfill. Bioremediation is, in many situations, a more cost-effective approach than containment or treatment by traditional chemical, physical, or thermal processes. For complex mixtures of waste, biodegradative treatment offers a typical savings of 60 to 90% over landfill disposal costs. Accurate financial comparisons between waste-treatment techniques require analysis of specific processes and specific waste compositions. Whether the method is biological or nonbiological, the possibility of incomplete remediation exists. Toxic by-products or residues of primary treatment (incinerator fly ash, chemical sludge, spent filters and scrubbers, etc.) may themselves be subject to a final disposal step. Such costs must be included in

comparisons. Since bioremediation can ideally destroy organic wastes without creating adverse residues, such considerations may favor bioremediation over nonbiological alternatives.

Bioremediation and Biodegradation

Environmental bioremediation, as noted above, refers to a process of environmental improvement in which biological organisms or products play a key role (2–4). Many microorganisms can adapt their catabolic machinery to make use of undesirable environmental pollutants as food sources, thereby degrading the environmental pollutant from an energy-rich state to an energy-poor one. Thus, microbes bioremediate the environment as they biodegrade the pollutant to obtain energy. The terms bioremediation and biodegradation are sometimes erroneously treated as synonymous. Biodegradation specifically refers to chemical breakdown or mineralization of materials (not necessarily waste) facilitated by biological organisms or products. The results of this action may or may not be judged to be remediative, although biodegradation of waste often leads to bioremediation. Biodegradation of organic chemicals occurs enzymatically. Catabolic enzymes are proteins that catalyze specific degradation reactions of organic molecules and usually display high affinity for certain substrates. By this process, organic wastes (and some inorganic wastes) may be absorbed by the microbe and internally broken down, or wastes may be degraded externally by secreted enzymes, after which the metabolites are absorbed and utilized. Alternatively, microbial catabolic enzymes, produced for the breakdown of normal food sources, may degrade certain wastes that are present as well. This process, termed co-metabolism, requires an ample supply of the preferred food substrate and also requires that the degrading enzymes come into contact with the waste by waste absorption or by enzyme secretion. Either direct metabolism or co-metabolism can be enhanced in rate and extent over that which occurs naturally by measures that boost the microbial population or increase the availability of food and/or growth-limiting nutrients. The basic information required to enhance natural biodegradative processes is knowledge of the microorganisms present in a given site, their growth requirements, and how these organisms interact with one another and their environment.

General Considerations

Bioremediation applies appropriate microbes or higher organisms to undesired contaminants and manipulates conditions to maximize the desired activity. Non-biological methods have been effectively combined with biological methods to enhance degradation of recalcitrant pollutants. For example, chemical peroxidation of organohalogenes has been demonstrated to increase bacterial degradation dramatically (99). No single method can be expected to work equally well on all constituents of a diverse mixture; a combination of remediation approaches may represent the most cost-effective solution among several practicable ones.

The methods most appropriate for concentration and containment of pollutants depend greatly on water content. Traditional approaches have involved incineration and wet oxidation with controlled emission in order to capture the contaminants. When the water content is high, these methods do not work well and contaminant concentration by bioaccumulation may be an attractive alternative. *In situ* bioaccumulation by certain plants is a promising technology for dealing with slowly leaching metal sources such as mine drainage. The bioaccumulating organisms become loaded with toxic substances, and the resulting biomass, whether it is bacterial or plant, must be collected and disposed of before it decays. Bioremediation of radioactive pollutants is a developing field of great importance for the future acceptance of nuclear power. Reviews of the interactions between radioactive substances and microorganisms and microbial products have been published (77,100,101). Radioactive metals may potentially be concentrated and reprocessed in the same ways as ordinary heavy metals.

Categories of Bioremediation Technology

Three categories of bioremediation techniques have been identified. They involve distinct technologies for remediation *in situ* and for use of biofilters and bioreactors. The first category, *in situ* land treatment—treatment of contaminated material in place—is a method for bioremediation of contaminated soil and, to some extent, of associated groundwater. The action may be as simple as nutrient enrichment or may involve further manipulation of site conditions, such as inoculation of the contaminated site with selected microorganisms, mixing and aeration of surface soils, or pH

modification. *In situ* treatment involves a minimum amount of effort and cost since the contaminated material does not have to be contained or extensively handled. The expensive and potentially dangerous job of transporting waste is eliminated, and further disturbance of the site itself is minimized. The chief drawback to this approach is that degradation monitoring is difficult, and concerns about toxicant dispersal may rule out such methods altogether. A related bioremediation approach, referred to as land farming, is applicable to solids, sludges, liquids, or contaminated soil that is treated at a designated site. The wastes are sprayed onto, plowed into, or otherwise mixed with surface soils; aeration, water, and nutrient enrichment are applied to achieve the best conditions for microbial and/or plant growth. Land farming is identical in practice to *in situ* treatment of contaminated soil, except that treatment takes place at a different location from the site of contamination. Waste transport allows the use of a dedicated remediation facility where various considerations, such as containment, monitoring, accessibility, security, etc., can be optimized. In most cases, however, land farming as a final treatment for wastes is banned in the United States by the terms of the 1984 Hazardous and Solid Waste Amendments and the 1976 Resource Conservation and Recovery Act (RCRA). The so-called RCRA land ban prohibits any form of waste disposal on land unless the waste has been treated to prescribed standards. Thus, land farming can currently be used only as an intermediate treatment step to be followed by further treatment or disposal (102). As with *in situ* land treatment, land farming requires large amounts of dedicated land and usually months or years before bioremediation is complete.

The second category of bioremediation consists of biofiltration methods for treating gaseous streams. The usual biofilter design is a permeable organic filter bed, which serves as a culture medium for microorganisms. Nonliving immobilized enzyme biofilters are also possible biofilters. Biofilters may concentrate pollutants in a manner analogous to conventional stack gas scrubbers or may be used in some cases to biodegrade contaminants to a nontoxic form that is compatible with natural environmental processes. The latter holds the advantage of actual reduction of waste rather than simply repackaging for later disposal. Such a biofilter can be thought of as a type of biological catalytic converter. As a general rule, operator intervention is

minimal. When accumulating waste levels become toxic to the microbes or when nutrients from the organic medium are depleted, the filter bed is changed. This is the only biological technique currently available to remediate airborne pollutants. Application of biofilter technology is at present limited to removing volatile organic compounds (VOCs) from point sources only. Waste remediation technologies for treatment of ambient air pollution are essentially nonexistent.

Bioreactors are the third and most technologically sophisticated category of environmental bioremediation. Bioreactors offer a much faster means of waste biodegradation than land treatment and more control over reaction conditions and effluent quality than simple biofilters. In contrast to the months or years required for land treatment, bioreactors may require only days or weeks for effective degradation of specific pollutants. Slurry-phase bioreactors are suitable for remediation of high concentrations of soluble organic wastes in soil or sludges; these reactors are recognized as capable of handling up to at least 250 g/kg levels of organic wastes (103). Recalcitrant wastes, such as organohalogenes, may require pretreatment; some wastes, such as organic corrosives and most inorganics, may not be degradable by current bioreactor systems.

There are many varied bioreactor designs, which allows for treatment of a wide variety of wastes with varied water and organic content. In some designs bacterial growth is optimized in a well-mixed aqueous phase contained in a lagoon, tank, or other reactor vessel into which slurries of waste or contaminated material are introduced. Other bioreactor designs specifically limit mixing. Control over mixing, aeration, temperature, nutrient levels, water content, etc., is increased dramatically over that which is possible with land treatment techniques. Degradation monitoring is made easier because the system is contained and output is regulated. Furthermore, control over release of nonindigenous organisms to the environment is possible. The bioreactor approach is more expensive than *in situ* or land treatment methods because specialized reactors and equipment are utilized and materials must be more extensively handled and sorted. Bioreactor operation requires much attention and expertise to achieve the rapid degradation that offsets the higher costs (7). Maintenance of optimum conditions for microbial activity is far from trivial.

Additionally, variations in reactor conditions, e.g., oxygen concentration, temperature, and pH, can affect not only the rate of degradation, but also the identity of the metabolites produced. In contrast to land treatment, bioreactors require better knowledge of both the exact composition of the waste to be degraded and the degradation pathways catalyzed by the microbes in use. Portable bioreactor units permit a type of *ex situ* bioreactor treatment with minimal transportation costs. This technique has perhaps the greatest potential for use as a routine treatment method for industrial wastes. Waste effluent streams can be treated at the site of production by directing them into a bioreactor process line.

In aqueous solution, dispersants that increase the bioavailability of organic wastes to microbes may also be employed. In many cases the degradation process proceeds rapidly at first, then slows over time. This rate change may be explained by product inhibition of microbial action or by the presence of waste components that are tightly bound on or within soil particles. Surfactants and other chemicals that accelerate desorption can be used in bioreactor systems to increase the degradation of tightly bound materials. For more in-depth treatment and discussion of other considerations of reactor dynamics see Weber and DiGiano (7).

Applications of Bioremediation Methods

Enhanced soil bioremediation may be achieved as simply as adjusting nutrient, pH, water, and oxygen levels to encourage growth of indigenous soil microbes by fertilizing, irrigating, and tilling the soil. Tilling to achieve greater mixing between contaminants and microbes can become an elaborate process if large areas are involved. In some cases, forced aeration of surface soils with compressors or vacuum pumps may be applied. Extensive characterization of soil and waste parameters can be used to determine exactly what enhancement measures are necessary or if bioremediation is feasible (104,105). Inoculation of soil with microbial strains selected for their particular abilities may appear to offer the greatest potential for desired biodegradative action. In many cases, such techniques prove ineffective due to incompatibilities between microbes and environmental conditions or due to technical limitations on inoculant size (106,107). Unwanted surprises may also occur, such as growth of a variant opportunistic pathogen that grows

particularly well on a common organic contaminant, e.g., gasoline (108).

Bioreactors for remediation of contaminated soil offer advantages in enhancing the transport of both nutrients and waste to the degrading microorganisms. Furthermore, bioreactors can be completely contained and all outputs regulated, including gaseous emission of volatile wastes and metabolites. Soil slurries can be oxygen saturated for aerobic degradation or anoxic for anaerobic degradation, as desired. Organisms can thus be utilized in bioreactors that would never thrive or even survive in some soil environments. Because of the containment this method affords, it is the most likely bioremediation procedure to make use of genetically engineered microbes.

Selected or genetically engineered microbes that produce the high-affinity metal-binding protein metallothionein have been suggested as a biological metal-sequestering system for waste water treatment (109). Production of metal-binding proteins by cultured microorganisms for subsequent extraction is also potentially useful for treatment of metal-contaminated wastes and environments. Immobilized metallothioneins could be used in biofilters and bioreactors to remove dissolved metals from waste streams.

Contaminated subsurface aquifers frequently accompany soil contamination. Bioremediation of groundwater resources presents unique problems and risks. Among the most obvious of these problems are that groundwater is mobile, whereas soil is generally stationary, and that people and livestock frequently drink untreated groundwater. Thus, there will often be an additional urgency factor associated with groundwater cleanup that may justify more drastic and expensive measures.

The usual approach in remediation of contaminated aquifers is groundwater pumping and surface treatment to eliminate the water-soluble wastes. The treated water is then recharged into the aquifer via one or more injection wells at some point upgradient to the contaminated zone. Pump-and-treat operations can incorporate bioremediation in at least two ways. The most obvious method uses biological (bioreactor) surface treatment; but, like any pump-and-treat approach, this method is only able to degrade wastes in the mobile, aqueous phase. It is important to recognize that many organic wastes have low water solubilities, and aquifer-associated soils will often contain larger volumes of organic wastes than the water itself. The conviction

that pump-and-treat measures can be effective has led to an appreciable effort in this direction at numerous hazardous waste sites. However, the goal of remediating aquifers to drinking water standards by such techniques may be unrealistic in many, if not most, cases. Curtis C. Travis, director of the Center for Risk Management and head of the Risk Analysis Section of the Health and Safety Research Division at Oak Ridge National Laboratory, Oak Ridge, Tennessee, recently addressed the question of whether it is technically possible to restore contaminated groundwater to an environmentally sound condition (110). He strongly argues that pump-and-treat technologies cannot do the job. Contamination levels at remediation sites are typically two to three orders of magnitude above allowable drinking water limits. Based on practical experience gained in pumping and treating contaminated aquifers over the past 10 years, Travis argues that treatment typically drops pollutant concentrations by a factor of 2 to 10, then levels out with no further decline. Cessation of pumping is often followed by a rebound in aquifer waste concentrations. The problem is largely that sites are typically contaminated with organic wastes that do not readily dissolve in the aqueous phase. The waste either remains adsorbed to the soil matrix, floats on the top, or sinks to the bottom of the water table. Therefore, wastes only slowly seep into the groundwater at a diffusion-limited rate, and cannot be significantly changed by groundwater pumping. Pump-and-treat measures may dramatically reduce pollutant concentration in the aqueous phase of the aquifer; when the pumps are switched off, however, pollutants gradually leach out of the soil and the aqueous concentration rises again. Many leading hydrologists have concluded that hundreds to thousands of years of pumping could be required to purge some contaminated aquifers of their organic waste contaminants (CW Hall, unpublished data). Travis draws the unfortunate conclusion that "Not a single aquifer in the United States has been confirmed to be successfully restored through pumping and treating" (110). The implication is that, although pump-and-treat measures may be useful to limit dispersal of a waste plume into the water table, massive excavation of soil is usually required to remove the source of the problem.

A more recently developed bioremediation approach to water treatment is subsurface *in situ* remediation. The treated

water can be nutrient- and oxygen-enriched prior to recharge, stimulating aerobic biodegradation of soil-bound, water-insoluble wastes by indigenous soil microorganisms. Actual oxygen content of the water can be boosted by air pumps, or alternative oxygen sources such as hydrogen peroxide may be added. Surfactants and other organic waste desorbing chemicals can also be added to increase waste bioavailability. If a surface bioreactor is used, some portion of the active microbial biomass can be recharged with the water, providing continuous inoculation of the contaminated aquifer and soil. Although stimulation of aerobic metabolism is the objective of most systems, the reinjected groundwater can be enriched with nitrate to stimulate growth and enhance the biodegradative action of anaerobic denitrifying microbes. Recently, this approach has proved effective in degrading the various organic constituents of gasoline, with toxicity reductions comparable to those seen in aerobic degradation (111). As with *in situ* soil treatment, the success of subsurface aquifer bioremediation is largely determined by waste and soil characteristics. Soil permeability is especially important to the success of nutrient enrichment and inoculation efforts.

Over the years, a number of lakes and rivers have become seriously contaminated with various industrial wastes in many parts of the United States. In some cases, the sources of pollution have been reduced or even eliminated. Public demand for remediation to a condition safe for fishing and other recreational uses is growing. Unfortunately, technologies for surface water remediation are not nearly as well developed as those for soil or even groundwater. Part of the problem lies in the size of many bodies of water. It is technically and environmentally impractical to divert a large flowing body of water from its course for treatment. Also, as with underground aquifers, the water contamination problem is largely a sediment contamination problem. Many persistent wastes become tightly bound to bottom sediments from which they slowly leach out, and thus cannot readily be removed by water treatment. Conventional treatment typically involves dredging and removing bottom sediment in the most polluted areas, but such measures can themselves be environmentally devastating and there is a risk of remobilizing toxicants accumulated over many years. Furthermore, the excavated sediment must still be treated and/or disposed of as toxic

waste. Workers are turning to *in situ* bioremediation almost as a last resort.

Surface-water bioremediation technologies are largely being developed in place. An ongoing example is the General Electric (GE) site in Fort Edward, New York, on the upper Hudson River. For many years GE legally released PCBs into the river from a plant that manufactured capacitors. When PCBs became priority environmental pollutants in the early 1980s, at least 20 miles of the river bottom were found to be contaminated downstream of the plant. GE began looking for remediation options. In 1991, GE conducted an extensive field research program to characterize natural degradation of PCBs at this site (112) and discovered that the indigenous consortia of microorganisms was exceptionally good at degrading PCBs. Presumably, since the PCBs have been present in this site for a significant time period (at least 35 years), the indigenous microorganisms have adapted to utilize the material as a food source. Both anaerobic and aerobic biodegradation have been identified as part of the natural process of remediation, which can be slow. Field tests in cylindrical caissons sunk into the river sediment at this site have identified the variables that can be manipulated to enhance *in situ* biodegradation of PCBs. The addition of inorganic nutrients, the organic co-metabolite (biphenyl), and oxygen significantly increased PCB degradation rates. Addition of selected PCB-degrading bacterial cultures did not dramatically improve biodegradative efficiency. No more than 60% of the PCBs was degraded in any laboratory or field experiments, a finding attributed to tight sediment adsorption of the least water-soluble PCB compounds (113). More information on degradation rates, products, and variability under natural conditions is required for a realistic evaluation of the role that bioremediation may play in this and other surface-water sites contaminated by organic waste.

Humans have long exploited the volume-dilution power of the sea to dispose of unwanted wastes. Although concern about waste accumulation in marine environments is increasing, especially for coastal waters, marine remediation efforts are nearly nonexistent. The notable exception to this rule is crude oil and refined petroleum product spills. Tanker spills account for only 13% of the estimated 3.2 million metric tons of annual marine petroleum hydrocarbon inputs (114). Yet tanker spills have remained the focus of research efforts

related to remediation of marine oil contamination. The potential for truly massive spills from modern supertankers, and the readily visible direct impact on affected areas, have captured the public's attention and sensitized regulatory and industry groups to the local destructive potential of such accidents. Petroleum is a complex mixture of thousands of individual compounds, and the degradation pathways of spilled oil are numerous and complex. Biodegradation, especially by microbes, is believed to be one of the primary mechanisms of ultimate removal of petroleum hydrocarbons from marine and shore environments (114,115). Acceleration of this natural process is the objective of bioremediation efforts. Bioremediation has yet to become an established spill-response technology, but some attempts to implement it have been encouraging. The inability of established nonbiological techniques to cope with recent large spills has led to increased interest in bioremediation. Special problems associated with marine oil spills include the uncontained nature of the waste, the potential size of the contaminated area, and difficulty in access for remediation and monitoring activities.

As with other forms of *in situ* bioremediation, natural biodegradation of marine oil spills may be enhanced by inducing changes in either the microbial population or the availability of microbial nutrients. Most researchers have concluded that nutrient availability is the chief limitation of natural biodegradation and most research has been directed toward enhancing nutrient availability (116). Marine oil-spill cleanups represent some of the largest *in situ* remediation projects ever attempted. The March 1989 spill of 11 million gallons of crude oil from the supertanker Exxon Valdez into Prince William Sound, Alaska, provided a testing ground for many nutrient enrichment technologies. The U.S. EPA and Exxon spent about \$8 million on a joint program to test and apply such measures (117). Open-ocean nutrient enrichment is problematic. Physical dispersion of oil and nutrients makes both application and assessment difficult. The impossibility of maintaining discrete treated and control areas of a floating slick makes any evaluation of success suspect. In Prince William Sound, therefore, the beaches became the primary bioremediation target. The microorganisms present in the beach sediment were considered diversified and sufficiently capable to complete the oil biodegradation desired. Following the appraisal of small

scale tests, Alaskan beaches received large scale nutrient applications. Eventually, about 110 miles of beach were treated with many tons of nutrient materials. Both water soluble and oleophilic fertilizers were effective, with visible clearing of oiled beaches evident in many cases. The results obtained indicate that, for the conditions encountered, the bioremediative action of indigenous bacteria can safely be accelerated 2- to 4-fold over control beaches by a single addition of nutrients. A second application 3 to 5 weeks later boosted this figure to as high as 5- to 10-fold (116). Analysis of the process and steps involved in this bioremediation, and final assessment, are still underway. Assessments of inoculation or seeding oil spills with selected microorganisms have, thus far, been inconclusive (118,120).

The greatest theoretical problem with bioremediation as a first response to oil spills is the time of action. Movement of oil slicks toward sensitive areas (i.e., coastal wetlands, beaches, shellfish beds, etc.) may necessitate a rapid nonbiological response, such as chemical dispersal or burning, to prevent contamination. In such cases, bioremediation may prove useful as a secondary treatment option or when paired with nonbiological methods of degradation enhancement. A proposed first response to oil spills utilizes titanium-dioxide coated floating glass microbeads to catalyze photooxidation of the oil (119,120). When these beads are applied to a floating slick, a microfilm of oil coats the beads, and the semiconducting titanium dioxide compound absorbs sunlight energy, thus catalyzing the oxidation of the organic microfilm (121). The resulting breakdown products are more water soluble and more bioavailable, which results in more rapid biodegradation.

Another emerging application of bioremediation, with potential yet to be fully realized, is biodegradation and/or removal of environmentally undesirable compounds from air through biofilter technology. This technology has been shown, primarily by groups working in Europe, to be suitable for large-scale biodegradation of VOCs [(122); AJ Dragt and SPP Ottengraf, unpublished data]. The idea is to direct the flow of a VOC-laden gas stream through a bed containing mixed cultures of microorganisms that mineralize the VOCs. The actual filter bed is a wet, biologically active layer of compost, peat, or soil, which provides both a structural support matrix and a source of inorganic nutrients to the microbial population. Inert structural additives,

such as plastic beads, may be included to decrease flow resistance and reduce structural changes due to aging. Compost mixtures made from municipal wastes, wood chips, bark, or leaves have usually been the media used in European gas biofilters. U.S. designs have typically utilized soil beds, which suffer from lower biodegradation capacity, larger space requirements, and higher gas flow resistance (122). Given sufficient retention time in the filter, the organic contaminants diffuse into the aqueous biolayer (or biofilm) surrounding the filter-bed particles, where they are degraded by microbial metabolism. The final products are microbial biomass, resulting from growth of the microorganisms, and mineral end products. Volatile end products will be carried out in the gas effluent stream. Nonvolatiles will accumulate in the filter media, which eventually must be changed. Naturally occurring microorganisms are usually present in quantities adequate to handle easily biodegradable compounds like alcohols, ethers, simple aromatics, etc. More degradation-resistant chemicals, such as nitrogen- and sulfur-containing organics and especially chlorinated organics and aliphatics, may require inoculation with selected strains of microbes to achieve desired degradation efficiencies. Although every application must be evaluated individually, biofilter technology represents a VOC abatement option that is competitive in many cases on both an efficiency and a cost basis (122). As many as 500 aerial biofilters may be functioning in Germany and the Netherlands, ranging from small, simple systems associated with farms and food-processing plants to large-scale facilities at chemical plants, foundries, print shops, and paint shops. Biodegradation efficiencies in excess of 90% have been achieved in a number of industrial installations (AJ Dragt and SDP Ottengraf, unpublished data).

Aerobic versus Anaerobic Metabolism of Wastes

For purposes of bioremediation, aerobic microbial metabolism has traditionally been the focus of attention. Aerobic degradative pathways in microbes and in animals break down organic molecules oxidatively by using divalent oxygen or other active oxygen species, such as hydrogen peroxide, as electron acceptors. Aerobic catabolism of organics ultimately results in familiar mineral products—carbon dioxide and water. Aerobes are capable of degrading most organic wastes, provided enough oxygen is

available. Some compounds, notably the organohalogenes, are highly resistant to aerobic biodegradation (termed recalcitrant or persistent wastes). Resistance of most aromatic and aliphatic compounds to degradation is dramatically increased by halogenation (most commonly chlorination); further halogenation results in increased resistance (102).

Anaerobic microbes degrade organics reductively, eventually resulting in the mineral end product methane. In the case of carbohydrate compounds, carbon dioxide and free hydrogen also are produced. Although they are not usually utilized for routine waste degradation, some anaerobes are very adept at dechlorination of common recalcitrant organochlorine compounds, notably PCBs, organochlorine pesticides, such as DDT, and chlorinated aliphatics, such as the industrial solvent trichloroethylene (TCE) (86,123,124). Thus anaerobic microbial catabolism (sometimes called fermentation) offers a bioremediation option to deal with persistent wastes. Complete anaerobic degradation of wastes, however, may be slow.

The major problem with anaerobic digestion of organochlorine wastes is that biodegradation is often incomplete (at least on a practical time scale) and may result in toxic metabolites. The use of mixed cultures containing both aerobes and anaerobes facilitates mineralization of many organochlorines (125). In practice, a sequential bioreactor system utilizing both anaerobic and aerobic reactors could be employed. For example, PCBs or chlorinated aromatics could be dechlorinated anaerobically, then fed into an aerobic bioreactor to be fully mineralized to carbon dioxide and water. Similarly, TCE and perchloroethylene may be reductively metabolized to vinyl chloride (a toxic chemical), which can then be subjected to aerobic biodegradation. Commercial versions of such two-stage hybrid bioreactor systems are currently under development (117). Isolation and characterization of dehalogenases (dehalogenating bacterial enzymes) for possible development of immobilized enzyme reactors and biofilters are also being conducted (126).

Counting Time, Dollars, and Risks

For a given remediation project, it is likely that several bioremediation options exist. The most obvious constraint on any remediation effort is cost; the business of waste remediation can be very costly. Costs may

be expected to decrease as techniques and equipment are improved and become more standardized. Cost estimates for various technologies may be obtained from contractor-supplied values included in the U.S. EPA Vendor Information System for Innovative Treatment Technologies database (U.S. EPA, Research Triangle Park, NC). Costs tend to be highly dependent on specific properties of a given site, and so-called typical values may not be meaningfully applied to specific case-cost projections.

Bioremediation often occurs through unassisted natural biodegradation, albeit slowly. If consideration of time scale were eliminated, the “do nothing” approach might be best and has been suggested as the optimum overall remediation option in certain types of contamination, such as marine oil spills (127). Even so, political and legal considerations often effectively eliminate the “do nothing” option. However, cost factors are likely to result in the selection of something other than the fastest option.

Risks to human or environmental health associated with a particular remediation scheme often play a major role in selection of the most desirable technique. Examples include the risk of waste dispersal, as when surface spills threaten groundwater quality, the risk of transportation of toxic wastes through populated or environmentally sensitive areas, or the risk of accidental release of genetically engineered organisms.

Appreciation of the potential of natural systems to regulate levels of aquatic toxicants has led to the development of constructed wetlands for bioremediation of complex wastes. It has been observed that wetlands have a buffering ability on surface waters with respect to circulating nutrient and pollutant levels. Wetlands have the capacity to store excess nutrients or wastes and to release stored excesses under the right environmental conditions [for a collection of reviews on this subject, see Hammer (128)]. A constructed wetland is an artificial habitat, most visibly made up of vascular plants and algal colonies, which also provide a structural and nutritional support for an associated, highly heterogeneous microbial community. One of the most promising applications of constructed wetlands is for *in situ* bioremediation of metal contamination. It is not always known to what extent the observed metal removal in natural wetlands is due to bacterial action and what is due to higher plant or algal activity. In any case, many of these organisms exist in a symbiotic

arrangement, and multitrophic cultured systems are increasingly being viewed as an alternative to monocultures or even heterogeneous bacterial cultures. Field tests on acid mine drainage effluent have indicated that such systems are capable of removing metals via multiple pathway biological action (129). The use of both natural and constructed wetlands for heavy metal abatement is of great potential value, but questions remain about the eventual fates of the metals. Some means of extraction, such as removal of plant or sediment material, is necessary to prevent remobilization of metals from dead organic material or trophic transfer to grazing animals.

Aerial biofiltration is a developing technology that has yet to see widespread application in the United States. It has received extensive attention in this review because it is the only proven biological system that can degrade airborne pollutants. At present, it is an emission control technology applicable only to pollutant point sources. Whereas no remediation technique has yet been established to remove ambient levels of organic pollutants from air, extensions of biofilter technology may yield such techniques in the future. Potential technical problems include maintenance of degradation efficiency with variable off-gas flow rates, accumulation of damaging metabolic by-products in the filter bed (inorganic acids from sulfur, nitrogen, and chloride-containing wastes, for example), and the possibility of release to the atmosphere of undesirable amounts of microbial or fungal organisms or spores.

Environmental Diagnostics and Surveillance Needs

Bioremediation is an emerging field, the full potential of which is as yet unknown. There is a tremendous need for further basic research and development, especially in the areas of environmental site and waste diagnostics, waste-technology matching, and integration of multiple remediation techniques.

There are two methods traditionally used to monitor bioremediation. The most common approach monitors pollutant and metabolite concentrations as an indirect measure of the extent of biological activity that has occurred. The more rigorous approach is to directly monitor the bacterial population. Modern methods of molecular biology offer alternatives to the tedious and time-consuming classical culture-and-identify methods. Gene-probe methods that directly identify the microbial species

present in a given soil or sediment sample are in development (91). Gene probing can also give an indication of the relative natural abundance of organisms with the potential to degrade specific pollutants at a given site.

There is a clear need for improved methods of environmental surveillance for the prevention of adverse environmental conditions. Continued development of new methods, including lab-bench assays and gene-probe technologies and their utilization, may provide some of the desired information and early warning for environmental hazards. When required, bioremediative approaches need to be applied with the understanding that each local environment requires individual attention and detailed site evaluation. In bioremediation of a contaminated area, performance feedback to researchers with regard to the transport, fate, and possible toxicity of the metabolites produced is of tremendous value for method refinement. Moreover, the site evaluation processes must incorporate expertise from those knowledgeable in other remediation technologies as well as bioremediation experts. Coupled and integrated methods of containment, destruction, and biodegradation of pollutants are certain to yield more cost-effective cleanup solutions than procedures that focus on a single remediation technology.

The primary limitation to the widespread use of many bioremediation approaches is often the extent to which the pollutant is available to the microbial population. The bioavailability of many chemicals diminishes with time as a result of weathering and aging phenomena, and the time window in which appropriate bioremediation technologies can be employed requires further definition. Many organic pollutants do not readily enter the bioactive, aqueous phase of soil and sediment environments. Their bioavailability to the microbial population might be appreciably increased by the use of appropriate surfactants, dispersants, chelators, or emulsifiers. The physical matrix in which pollutants are found largely determines the rate at which the pollutants become bioavailable. Recognized areas for research advancement include alteration of sorption and desorption kinetics at contaminated sites to enhance the rate of bioremediation. Improved methods to make pollutants more bioavailable and an increased knowledge of the critical parameters involved are clearly needed.

A major recommendation of a U.S. EPA bioremediation workshop (130) was

that innovative and novel processes for dealing with complex waste mixtures be explored. Nondegradable wastes in such mixtures, notably heavy metals, can be extracted from mixed-waste streams by biochemical action and may be concentrated by the method of bioaccumulation whereby wastes are absorbed and stored in the tissues of the organisms utilized. Improved bioremediation of complex mixtures might take advantage of the fact that microbes can be selected to mobilize, immobilize, or fix compounds or ions in such a way that they are rendered susceptible to further treatment. The first stage of the process may require the action of a biodegrading, surfactant-producing or bioaccumulating organism. Development of practical means of exploiting such bioactivities under field conditions is a definite research need.

Improved methods for evaluating the degree of pollutant biodegradation in complicated environmental settings need to be developed, with simpler, less expensive, and more rapid assay procedures. Although complete mass-balance analyses are desirable, they are seldom obtainable. In light of this deficit, the durability and effectiveness of bioremediative technologies will require other indicators. New and better models for the processes involved, as well as improved diagnostic assays, may be required. If adequate models are developed, their use would greatly facilitate the design of cost-effective, safe, and practical bioremediative technologies to be applied in large-scale operations.

Conclusions

Effective bioremediation can be advanced and facilitated by making products that are readily susceptible to biodegradation. Keeping the principles of bioremediation in mind during product and process development is both prudent and profitable in the long term, as 3M (St. Paul, MN) and other leading international companies have shown (131). Unfortunately, long-term planning does not come naturally to everyone and there is great resistance to environmental protection and cleanup because of the effort and expense involved (132). Bioremediation is a technological attempt to exploit the abilities of microbes and other members of the biosphere to restore and maintain environmental quality for all forms of life in the ecosystem, especially humans. Education is important in achieving the widespread practices of prevention, recycling, and remediation for the purpose of improving future environmental health

and quality of life. Through education, societal customs can change in ways that both reduce and recycle wastes.

Perhaps the larger problem facing policy makers in the future is how to decide where available bioremediation dollars will benefit human and environmental health the most. For instance, in the last 15 years, more than 40,000 hazardous waste sites have been identified in the United States alone, but remedial efforts have been undertaken at only a few hundred sites and are largely restricted to the approximately 1300 sites on National Priority List (Superfund sites) (133,134). Target cleanup goals have been judged to be highly unrealistic in some cases (i.e., exceeding a 10,000-fold safety factor) (135). At most of these sites remedial

efforts are as yet incomplete, and some efforts have had little effect. In some cases, costs are astronomical, e.g., \$1 billion at the Rocky Mountain Arsenal near Denver, Colorado (136). Although there are cases where human health has been a legitimate concern (137), most cleanup decisions appear to be made in the absence of any evidence of adverse human effects (138).

Although the issues involved are undeniably complex, a considerably improved and enlarged remedial campaign would seem necessary to deal with all identified toxic waste sites in an adequate, responsible, and expeditious fashion. In the meantime, ineffective sewage treatment plants, septic tanks, and improper methods for dealing with farm animal waste are also of great practical concern; and these biologically

generated contaminants are also adversely affecting the health of undetermined numbers of people. It is clear that new bioremediation technologies that can better monitor and control many types of societal wastes are emerging. However, there is little incentive to find and develop new tools while relatively inexpensive chemical and bioremediation technologies that are proven and effective are unused. Water quality, which could benefit by new and existing bioremediative methods, can only get worse as populations grow. Priority setting, clearly stated environmental policies, and the implementation of appropriate remedial measures to deal with our contaminated land and water resources are badly needed.

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