BIOREMEDIATION

1. Introduction

Bioremediation is the process of judiciously exploiting biological processes to minimize an unwanted environmental impact; usually it is the removal of a contaminant from the biosphere. Like most definitions in biology, that of bioremediation is the subject of some debate. A narrow definition might focus on the conversion of contaminating organic molecules to carbon dioxide, water, and inorganic ions, and the oxidation or reduction of contaminating inorganic ions. A broader definition would include biological processes for ameliorating extremes of pH, concentrating contaminants so that they can be more easily removed by physical techniques, converting toxic species to less toxic or less bioavailable forms that pose less of a threat to the environment, and restoring functional ecosystems to contaminated or disturbed sites when the contaminants or disturbance cannot be removed.

The concept of "judiciously exploiting biological approaches" is also a subject of debate:

Some would restrict it to providing a nutrient that is otherwise limiting the most effective growth of organisms catalyzing the desired reaction, whether it is the degradation of an organic compound, the reduction or oxidation of an inorganic ion, or the accumulation of a contaminant. This simple approach has been successful with a range of contaminants. The nutrient might be a fertilizer providing nitrogen, phosphorus and other essential minerals, or an electron acceptor such as oxygen.

Others would extend the fertilizer concept to the simultaneous addition of readily biodegradable substrates along with the fertilizer nutrients to stimulate the growth of contaminant-degrading organisms most rapidly, and to aid in the rapid utilization of the fertilizer nutrients before they might be leached from the contaminated area. The specific requirements for the most efficacious substrates is an area of current research.

An alternative use of added readily degradable substrates is to drive the local environment toward anaerobiosis so that reactions such as reductive dechlorinations or reductive removal of nitro-groups are promoted.

A broader view would include the addition of a substrate to stimulate the growth of organisms known to degrade the contaminant of interest only as an incidental part of their metabolism, one might almost say serendipitously. This process is sometimes called co-metabolism, and it too has had success.

Others would include the addition of materials aimed at increasing the bioavailability of the contaminant to the degrading organisms. The most studied compounds are surfactants, but cations have been reported to increase the bioavailability of some organic compounds, and sorbents and clays are also considered. The dispersion of spilled oil on water by the application of dispersants is perhaps the major commercial use of this idea.

Another important option is the addition of remediating organisms. While the addition of contaminant-degrading bacteria has not yet had much documented success with natural products such as hydrocarbons, there is reason to expect that it will be efficacious with pollutants that are more recent additions to the environment. The planting of specific plants, in the process known as phytoremediation, is also a promising approach. Not only do the plants themselves have remediating activities, such as the accumulation of certain metal ions, but they also have extensive bacterial and fungal populations associated with their root systems, and inoculation of this rhizosphere is widely practiced. It is, thus, possible that planting seeds with microbial inoculants will become an option for bioremediation. There is much talk of genetically modifying organisms so that their remediative potential is increased, and in the future this may well become an important option.

The broadest view of "aiding and abetting" includes doing nothing, but merely watching natural processes occur without further intervention. This has been termed "Intrinsic Bioremediation", and it too has met with success. From an environmental point of view, although unfortunately not always from a regulatory viewpoint, it is important that any remediation intervention yield a clear net environmental benefit. Sometimes very mild stimulation of intrinsic processes may be the most environmentally responsible option.

Bioremediation overlaps some older biotechnologies. Municipal and industrial wastewater treatment is a well-established industry, and although it can be distinguished from bioremediation in that the pollutants are under physical control during treatment, the fundamental biological processes have much in common. Similarly composting is a well-established phenomenon, currently gaining popularity in the municipal solid waste treatment industry, and the biofiltration of waste gases is becoming a useful technology. Developments of these technologies, where the contaminant is already under physical control, will undoubtedly aid the development of bioremediation as an accepted tool for dealing with similar wastes when they have escaped control. This article focuses on biological treatments for contaminants when they have escaped into the environment.

Bioremediation is already a commercially viable technology, with estimates of aggregate bioremediation revenues of $2–3 billion for the period 1994–2000 (1). There are significant opportunities to enlarge upon this success. Bioremediation has applications in the gas phase, in water, and in soils and sediments. For water and soils, the process can be carried out *in situ*, or after the contaminated medium has been moved to some sort of contained reactor (*ex situ*). The former is generally rather cheaper, but the latter may result in such a significant increase in rate that the additional cost of manipulating the contaminated material is overshadowed by the time saved. Bioremediation may explicitly exploit bacteria, fungi, algae, or higher plants. Each, in turn, may be part of a complex food-web, and optimizing the local ecosystem may be as important as focusing solely on the primary degraders or accumulators.

Bioremediation usually competes with alternative approaches to achieving an environmental goal. Bioremediation is typically among the least expensive options, but an additional important consideration is that in many cases bioremediation is a permanent solution to the contamination problem, since the contaminant is completely destroyed or collected. Some of the alternatives technologies, such as thermal desorption and destruction of organics, are also permanent solutions, but the simplest, removing the contaminant to a dump site, merely moves the problem, and may well not eliminate the potential liability. Furthermore, by its very nature bioremediation addresses the bioavailable part of any contamination, and when biodegradation or bioaccumulation ceases this probably means that the bioavailable part of the contamination has been addressed. Residual concentrations of contaminants, although perhaps detectable by today's sensitive analytical techniques, may in fact have no residual environmental impact. The same cannot necessarily be said for nonbiological technologies, which may leave bioavailable contaminants at low levels.

Bioremediation also has the advantage that is can be relatively nonintrusive, and can sometimes be used in situations where other approaches would be severely disruptive. For example, bioremediation has been used to clean up hydrocarbon spills under buildings, roads, and airport runways without interfering with the continued use of these facilities.

On the other hand, bioremediation is usually slower than most physical techniques, and may not always be able to meet some very strict clean-up standards. Nevertheless, it is becoming a widely used technology. This article addresses bioremediation in its broadest sense, focusing on the contaminants that can be treated, the underlying biological processes that can mitigate the contamination, and the technologies that have been used, or are being developed, to treat them.

2. General Biological Aspects

The biosphere plays an important role in the great elemental cycles of the earth (2), and bioremediation must be placed in this context if it is to be appreciated in its broadest ramifications. One of the underlying fundamental truths of biological diversity is that if there is free energy available in the metabolism of a substrate, there is probably a guild of organisms that has evolved to make use of it. This is particularly germane to the biodegradation of organic molecules. For example, crude oil seeps to both land and water have occurred for millennia, and as a consequence, aerobic oil-degrading microorganisms are ubiquitous. If biology does not yet take advantage of a source of free energy, then it can be expected that there will be a strong selection pressure in favor of any organism that develops an ability to exploit it. This has been seen with by-products of nylon manufacture, where a *Pseudomonas aeruginosa* has gained the ability to degrade the novel compound 6-aminohexanoate linear dimer, a by-product of nylon-6 manufacture, as the sole source of carbon and nitrogen (3). The successful bioremediation of xenobiotic compounds, such as pesticides and herbicides, may well represent a similar acquisition of traits.

Not all organic molecules provide a source of free energy, however. Some, such as small halogenated solvents, provide no significant source of nutrients or energy, and their aerobic destruction can only occur co-metabolically with the degradation of a more nutritious substrate (4). The white-rot fungi provide another variation on this theme. These organisms seem unique in their ability to degrade lignin, the structural polymer of higher plants. They may not gain any direct energetic benefit from lignin degradation, but it clearly allows access to cellulose which is a substrate for growth. Lignin degradation is catalyzed by a group of extracellular peroxidases that generate nonspecific oxidants, and there have been several proposals to use these systems for destroying contaminating organic compounds (5).

With successful bioremediation, organic compounds can eventually be converted to carbon dioxide, water, and biomass. Similarly, nitrogenous molecules, such as excess ammonia or nitrate in ground water, can be mineralized to gaseous nitrogen. Alternatively they can stimulate the growth of plants, either terrestrial or marine, and the plant biomass can eventually be harvested so that the nitrogen is effectively removed from the local environment. Other nonorganic contaminants provide a different challenge for bioremediation. A few, such as mercury and selenium, are volatilized by some biological processes, but it is not clear that this is always beneficial. In some cases, such as chromium and arsenic, there is a dramatic difference in environmental toxicity depending on the redox state of the contaminant. Bioremediation has sometimes focused on this detoxification, usually by bacterial processes. A more satisfying approach would be to use a biological process to accumulate and concentrate the contaminant so that it can be removed for safe disposal. Fungi, algae, and higher plants have all been used in these efforts.

Table 1 explains a few of the biological terms that are widely used in discussing bioremediation, and which are used in the following text.

Table 1. **Some Biological Definitions Relevant to Bioremediation**

Term	Explanation
aerobic	conditions with free oxygen
anaerobic	conditions scrupulously free of oxygen
anoxic	conditions with very low levels of oxygen
autotrophic	growth using atmospheric CO_2 as sole source of carbon
co-metabolic degradation	biodegradation of a contaminant only fortuitously with degradation of a true substrate
denitrification	the reduction of nitrate to gaseous nitrogen
Eukaryotes	organisms with a membrane-bound nucleus; the protozoa, fungi, plants, and animals
eutrophic	very rich nutrient conditions, especially of nitrogen compounds
heterotrophic	growth at the expense of complex organic substrates
lignolytic	growth of white-rot fungi under conditions where they synthesize lignin-degrading peroxidases
methanogenic	very anaerobic conditions, where carbon dioxide is reduced to methane
methanotrophic	aerobic growth with methane as sole source of carbon and energy
mineralization	conversion of a contaminant to its simplest forms, eg, CO_2, H_2O, CH_4, N_2, Cl^-
nitrate-reducing, denitrifying	anoxic conditions, where nitrate is reduced to nitrogen gas
nitrification	the biological oxidation of ammonia to nitrite and nitrate
oligotrophic	very low nutrient conditions
Prokaryotes	organisms lacking a membrane-bound nucleus; the bacteria and archaea
recalcitrant	very resistant to biodegradation
reductive dehalogenation, reductive dechlorination	the sequential loss of halogen substituents under anaerobic, usually methanogenic, conditions
rhizosphere	the soil around plant roots; this zone has different microbial populations from the bulk soil
sulfate-reducing	very anaerobic conditions, where sulfate is reduced, by sulfate-reducing bacteria, to sulfide
vadose zone	the part of the soil above the water table

3. General Technological Aspects

Successful bioremediation hinges upon the effective application of the biology discussed above. Sometimes the contaminant is on the surface, so access to it is reasonably simple. Indeed the required technology may be as simple as broadcast spreaders or sprayers to apply fertilizers, or tilling the soil to allow good aeration. Of course this is not necessarily as simple as it sounds, since contaminated sites are often very different from agricultural fields, and the technology has to be significantly stronger to "plow" the soil. Frequently the contaminant is below the surface, and applying even simple bioremediation strategies can be very involved. Table 2 lists some of the technologies in use today.

Table 2. **Some Technological Definitions Relevant to Bioremediation**

Technology	Description
air sparging; aquifer sparging; biosparging	injection of air to stimulate aerobic degradation; may also stimulate volatilization
air stripping	injection of air to stimulate volatilization
aquifer bioremediation	*in situ* bioremediation in an aquifer, usually by adding nutrients or co-substrates
aquifer sparging	injection of air into a contaminated aquifer to stimulate aerobic degradation, may also stimulate volatilization
batch reactor	a bioreactor loaded with contaminated material, and run until the contaminant has been consumed, then emptied, and the process is repeated
bioactive barrier; bioactive zone; biowall	a zone, usually subsurface, where biodegrada tion of a contaminant occurs so that no contaminant passes the barrier
bioaugmentation	addition of exogenous bacteria with defined degradation potential (or rarely indigenous bacteria cultivated in a reactor and reapplied)
biofilm reactor	a reactor where bacterial communities are encouraged on a high surface area support, biofilms often have a redox gradient so that the deepest layer is anaerobic while the outside is aerobic
biofiltration	usually an air filter with degrading organisms supported on a high surface area support such as granulated activated carbon
biofluffing	augering soil to increase porosity
bioleaching	extracting metallic contaminants at acid pH
biological fluidized bed; fluidized-bed bioreactor	bioreactor where the fluid phase is moving fast enough to suspend the solid phase as a fluid-like phase
biopile; soil heaping	an engineered pile of excavated contaminated soil, with engineering to optimize air, water, and nutrient control
bioslurping	vacuum extraction of the floating contaminant, water, and vapor from the vadose zone; the air flow stimulates biodegradation
biostimulation	optimizing conditions for the indigenous biota to degrade the contaminant
biotransformation	the biological conversion of a contaminant to some other form, but not to carbon dioxide and water

Table 2 (*Continued*)

Technology	Description
biotrickling filter	a reactor where a contaminated gas stream passes up a reactor with immobilized micro-organisms on a solid support, while nutrient liquor trickles down the reactor
bioventing	vacuum extraction of contaminant vapors from the vadose zone, thereby drawing in air that stimulates the biodegradation of the remainder
borehole bioreactor; in-well bioreactor	the addition of nutrients and electron acceptor to stimulate biodegradation *in situ* in a contaminated aquifer
closed-loop bioremediation	groundwater recovery, a bioreactor, and low-pressure reinjection to maximize nutrient use, and maintain temperature in cold climates
composting	addition of biodegradable bulking agent to stimulate microbial activity; optimal composting generally involves self-heating to 50–60°C
constructed wetland	artificial marsh for bioremediation of contaminated water
continuous stirred tank reactor (CSTR)	a completely mixed bioreactor
digester	usually an anaerobic bioreactor for digestion of solids and sludges that generates methane
ex-situ bioremediation	usually the bioremediation of excavated contaminated soil in a biopile, compost system or bioreactor
fixed-bed bioreactor	bioreactor with immobilized cells on a packed column matrix
land-farming; land treatment	application of a biodegradable sludge as a thin layer to a soil to encourage biodegradation; the soil is typically tilled regularly
natural attenuation; intrinsic bioremediation	unassisted biodegradation of a contaminant
phytoextraction	the use of plants to remove and accumulate contaminants from soil or water to harvestable biomass
phytofiltration	the use of completely immersed plant seedlings, to remove contaminants from water
phytoremediation	the use of plants to effect bioremediation
phytostabilization	the use of plants to stabilize soil against wind and water erosion
pump and treat	pumping groundwater to the surface, treating, and reinjection or disposal
rhizofiltration	the use of roots to immobilize contaminants from a water stream
rotating biological contactor	bioreactor with rotating device that moves a biofilm through the bulk water phase and the air phase to stimulate aerobic degradation
sequencing batch reactor	periodically aerated solid phase or slurry bioreactor operated in batch mode
soil-vapor extraction	vacuum-assisted vapor extraction

4. Organic Contaminants

4.1. Hydrocarbons.

Constituents. Hydrocarbons get into the environment from biogenic and fossil sources. Methane is produced by anaerobic bacteria in enormous quantities in soils, sediments, ruminants and termites, and it is consumed by methanotrophic bacteria on a similar scale. Submarine methane seeps support substantial oases of marine life, with a variety of invertebrates possessing symbiotic methanotrophic bacteria (6). Thus, methanotrophic bacteria are ubiquitous in aerobic environments. Plants generate large amounts of volatile hydrocarbons, including isoprene and a range of terpenes (7). These compounds provide an abundant substrate for hydrocarbon-degrading organisms.

Crude oil has been part of the biosphere for millennia, leaking from oil seeps on land and in the sea. Crude oils are very complex mixtures, primarily of hydrocarbons although some components do have heteroatoms such as nitrogen (eg, carbazole) or sulfur (eg, dibenzothiophene). Chemically, the principal components of crude oils and refined products can be classified as aliphatics, aromatics, naphthenics, and asphaltic molecules. Representative examples are shown in Figure 1. The ratios of these different classes varies in different oils, but a typical crude oil might contain the four classes in a ratio of approximately 30:30:30:10. Most crude oils contain hydrocarbons ranging in size from methane to molecules with hundreds of carbons, although the lightest molecules are usually absent in oils that have been partially biodegraded in their reservoir. When crude oils reach the surface environment the lighter molecules evaporate, and are either destroyed by atmospheric photooxidation or are washed out of the atmosphere in rain, and are biodegraded. Some molecules, such as the smaller aromatics (benzene, toluene, etc) have significant solubilities, and can be washed out of floating slicks, whether these are at sea, or on terrestrial water tables. Fortunately the majority of molecules in crude oils, and refined products made from them, are biodegradable, at least under aerobic conditions.

Biodegradation. Methane and the volatile plant terpenes are fully biodegradable by aerobic organisms, and most refined petroleum products are essentially completely biodegradable under aerobic conditions. Estimates for crude oil biodegradability range up to 90% (8), and the least biodegradable material, principally polar molecules and asphaltenes, lacks the “oily” feel and properties that are associated with oil. These are essentially impossible to distinguish from more recent organic material in soils and sediments, such as the humic and fulvic acids, and appear to be biologically inert.

Numerous bacterial and fungal genera have species able to degrade hydrocarbons aerobically and the pathways of degradation of representative aliphatic, naphthenic and aromatic molecules have been well characterized in at least some species (8). Other organisms, such as algae and plants, do not seem to play a very important role in the biodegradation of hydrocarbons. It is a truism that the hallmark of an oil-degrading organism is its ability to insert oxygen atoms into the hydrocarbon, and there are many ways in which this is achieved. Figures 2 and 3 show the most well-studied. Once a hydrocarbon possesses a carboxylate or alcohol functionality it is almost invariably a readily degradable compound. A simple example at the human level is the difference between oleic acid, a high calorie

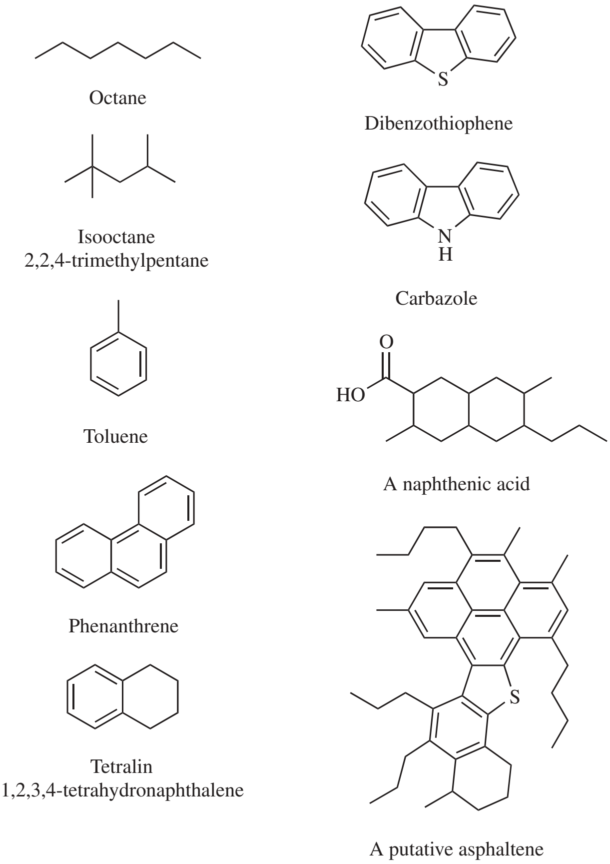


Fig. 1. Some representative hydrocarbons found in crude oil.

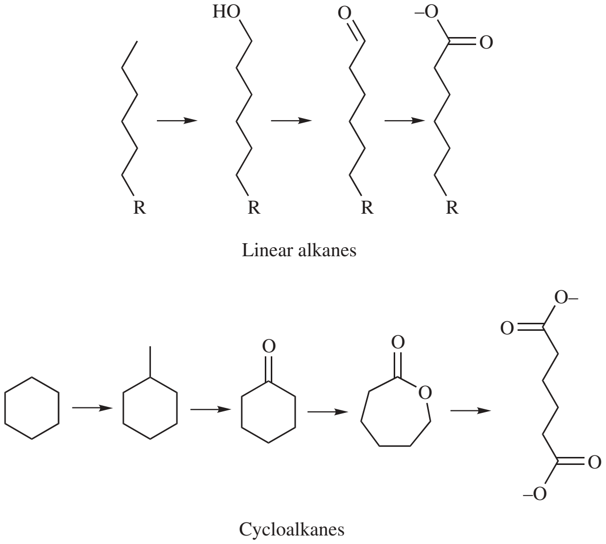


Fig. 2. Initial steps in the biodegradation of linear and cyclic alkanes.

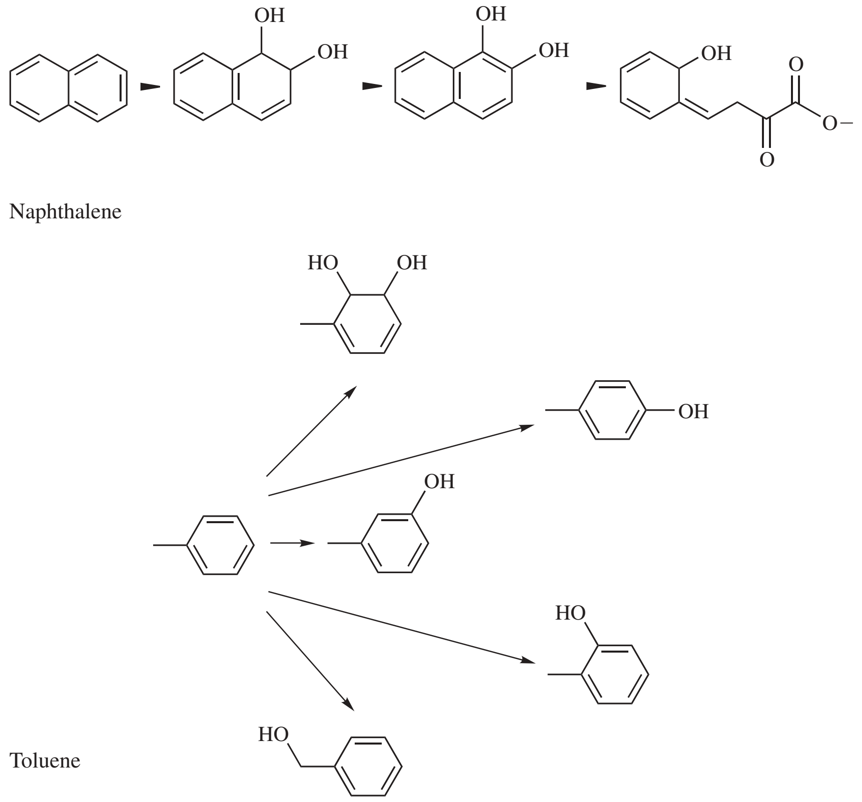


Fig. 3. Initial steps in the aerobic degradation of naphthalene, as a representative multiringed aromatic, and toluene. The different initial steps of toluene degradation are examples of the diversity found in different organisms.

food, and octadecane, present in mineral oil, which is so inert that it serves as an intestinal lubricant!

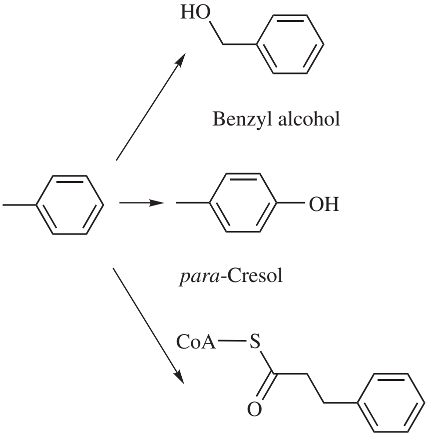
For many years it was assumed that oil biodegradation was an exclusively aerobic process, since any degradation must involve oxidation. Indeed the very existence of oil reservoirs indicates that anaerobic degradative processes in such environments must be very slow. Nevertheless, in recent years it has become clear that at least some hydrocarbons are oxidized by bacteria under completely anaerobic conditions, where the oxygen is probably coming from water. Limited hydrocarbon biodegradation has now been shown under sulfate-, nitrate-, carbon dioxide- and ferric iron-reducing conditions (Table 3). The phenomenon is still poorly understood, however, and at present the largest molecules demonstrated to undergo biodegradation under these conditions are hexadecane, heptadecene, and phenanthrene. The pathways of degradation are only beginning to be addressed. Figure 4 shows the intermediates identified in anaerobic toluene degradation in different organisms. It is noteworthy that while organisms capable of aerobic oil biodegradation seem to be ubiquitous, organisms capable of the anaerobic degradation of hydrocarbon have to date only been found in a few places.

Table 3. **Hydrocarbons That Have Been Shown to be Biodegraded Under Anaerobic Conditions**

Electron acceptor	Substrate
nitrate (to nitrogen)	heptadecene toluene, ethylbenzene, xylene naphthalene terpenes
iron(III) (to iron(II))	toluene
manganese(IV) (to Mn(II))	toluene
sulfate (to sulfide)	hexadecane, alkylbenzenes benzene naphthalene, phenanthrene
CO_2 (to methane)	toluene, xylene

Although the majority of molecules in crude oils and refined products are hydrocarbons, the U.S. Clean Air Act amendment of 1990 mandated the addition of oxygenated compounds to gasoline in many parts of the United States. The requirement is usually that 2% (w/w) of the fuel be oxygen, which requires that 5–15% (v/v) of the gasoline be an oxygenated additive (eg, methanol, ethanol, methyl *tert*-butyl ether (MTBE), etc). Although methanol and ethanol are readily degraded under aerobic conditions, the degradability of MTBE remains something of an open question. The compound was previously very rare in the environment, but now it is one of the major chemicals in commerce. At first it seemed that the compound was completely resistant to biodegradation, but complete mineralization has now been reported (9). Whether biodegradation can be optimized for effective bioremediation remains to be seen.

Bioremediation. Crude oil and refined products are readily biodegradable under aerobic conditions, but they are only incomplete foods since they lack any



β-PhenylpropionylCoA

Fig. 4. Proposed initial steps in the anaerobic biodegradation of toluene in different organisms.

significant nitrogen, phosphorus, and essential trace elements. Bioremediation strategies for removing large quantities of hydrocarbon must therefore include the addition of fertilizers to provide these elements in a bioavailable form.

Air. Hydrocarbon vapors in air are readily treated with biofilters. These are typically rather large devices with a very large surface area provided by bulky material such as a bark or straw compost. The contaminated air, perhaps from a soil vapor-extraction treatment, or from a factory using hydrocarbon solvents, is blown through the filter, and organisms, usually indigenous to the filter material or provided by a soil or commercial inoculum, grow and consume the hydrocarbons. Adequate moisture must be maintained for effective operation. Alternatively, trickling biofilters with recycled water are also in use. Both bacteria and fungi readily colonize such filters, and they can be very effective. Nevertheless, biofilters are usually equipped with a small granulated activated carbon "backup" filter to handle any sudden pulse loads that might overwhelm the biological capacity of the filter. Biofilters compete with granulated activated carbon filters, and are often cheaper because they minimize the cost of the granulated activated carbon, and the energy required to destroy the contaminant and the granulated activated carbon when the latter is saturated (10). Potential problems include plugging and uneven air or water flow, but successful designs work for many years with minimal maintenance except the occasional addition of nutrients and stirring of the bed.

Sea. Crude oil spills at sea are perhaps the most widely covered environmental incidents in the national and international media. Despite their notoriety, catastrophic tanker spills and well blow-outs are fortunately rather rare, and their total input into the world's oceans is approximately equivalent to that from natural seeps; significantly more oil reaches the world's oceans from municipal sewers (11). Physical collection of the spilled oil is the preferred remediation option, but if skimming is unable to collect the oil, biodegradation and perhaps combustion or photooxidation are the only routes for elimination of the spill. One approach to stimulating biodegradation is to disperse the oil with chemical dispersants. Early dispersants had undesirable toxicity, but modern dispersants and application protocols can stimulate biodegradation by increasing the surface area of the oil available for microbial attachment, and perhaps providing nutrients to stimulate microbial growth (12). Patents have been issued for dispersant formulations that specifically include nitrogen and phosphorus nutrients (13), but the products are not currently commercially available.

Bioremediation by the addition of oil-degrading microbes is often promoted as a treatment option for floating spills, but this approach has not yet met with any documented success (13).

Shorelines. The successful bioremediation of shorelines affected by the spill from the *Exxon Valdez* in Prince William Sound, Alaska, was perhaps the largest bioremediation project to date (14, 15). More than 73 miles of shoreline were treated in 1989 and similar amounts of fertilizer were used in 1990. Oil had typically penetrated into the surface gravel on these shorelines, occasionally getting as deep as 30 cm into the sediment. Since the gravel was typically very permeable, oxygen availability was unlikely to be the limiting factor for biodegradation, and indeed this was subsequently shown to be correct. Bioremediation thus focused on the addition of nitrogen and phosphorus fertilizers to partially remove the nutrient-limitation on oil degradation. Of course the addition of fertilizers was complicated by the fact that oiled shorelines were washed by tides twice a day. These tides would have rapidly removed any soluble fertilizer, so a strategy was sought that would provide nutrients for a significant length of time. Various approaches to applying fertilizers were tried, including both standard and slow release nutrients, oleophilic nutrients and solutions of liquid fertilizers. Two fertilizers were used in the full-scale applications; one, an oleophilic product known as Inipol EAP22 (trademark of CECA, Paris, France), was a microemulsion of a concentrated solution of urea in an oil phase of oleic acid and trilaurethphosphate, with butoxyethanol as a cosolvent. This product was designed to adhere to oil, and to release its nutrients to bacteria growing at the oil-water interface. The other fertilizer was a slow-release formulation of inorganic nutrients, primarily ammonium nitrate and ammonium phosphate, in a polymerized vegetable oil skin. This product, known as Customblen (trademark of Grace-Sierra, Milpitas, California), released nutrients with every tide, and these were distributed throughout the oiled zone as the tide fell. Fertilizer application rates were carefully monitored so that the nutrients would cause no harm, and the rate of oil biodegradation was stimulated between two- and five-fold (14,15).

A wide range of fertilizers, including agricultural and horticultural fertilizers, and bone and fish meals have been tried at the pilot scale, usually with at least modest success (13). Some current work is aimed at addressing whether providing a readily degradable substrate with the fertilizer nutrients helps immobilize the nutrients in biomass at the oiled site. Of course nutrient-supplementation is only likely to markedly stimulate the rate of biodegradation where nutrient levels are naturally low. It is unlikely that fertilizers will have a dramatic effect in situations where agricultural or municipal run-off maintains elevated levels of nutrients, such as happens in some estuaries and bays. Here aeration is likely to be most effective.

Bioremediation by the addition of oil-degrading microbes has been promoted as a treatment option for oiled shorelines, but this approach has not yet met with any documented success (13).

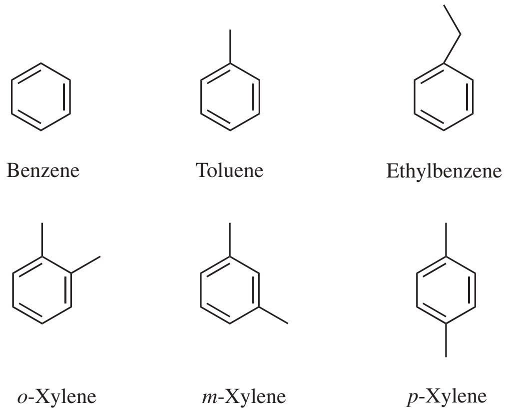
Areas where there are currently few remediation options include oiled marshes, mangroves, and coral reefs. These environments are generally easily damaged by human intrusion and physical cleaning options may not provide any net environmental benefit. Bioremediation may provide some attractive options, and some success has been claimed, on a small scale, with fertilizer applications (13). Marshes and mangroves offer the additional complication that they are typically anoxic. Perhaps the anaerobic degradation of oil could be stimulated by inoculation with anaerobic hydrocarbon degrading microbes, or perhaps gentle aeration or the addition of slow release oxygen compounds, such as some inorganic peroxides, might stimulate aerobic degradation without significantly changing the redox balance of these environments. This is an area where research is very much in its infancy and there are no well-documented success stories to date.

Bioremediation also offers options for dealing with oiled material, such as seaweed, that gets stranded on shorelines; composting has been shown to be effective.

Groundwater. Spills of refined petroleum product on land, and leaking underground storage tanks, sometimes contaminate groundwater. Bioremediation is becoming an increasingly popular treatment for such situations.

Hydrocarbons typically have a specific gravity of less than 1, and refined products usually float on the water table if they penetrate soil that deeply. In the parlance of the remediation industry, such floating spills are often called NAPLs (nonaqueous phase liquids). Indeed they are sometimes known as LNAPLs for light nonaqueous phase liquids, to distinguish them from more dense materials, such as halogenated compounds, which are more likely to sink in groundwater. Stand-alone bioremediation is an option for these situations, but "pump and treat" is the more usual treatment. Contaminated water is brought to the surface, free product is removed by flotation, and the cleaned water re-injected into the aquifer or discarded. Adding a bioremediation component to the treatment, typically by adding oxygen and low levels of nutrients, is an appealing and cost-effective way of stimulating the degradation of the residual hydrocarbon not extracted by the pumping. This approach is becoming widely used.

Hydrocarbons are not very soluble in water, but the most soluble components leach out of a spill if there is continual flushing. Typically only small aromatic molecules, the infamous BTEX (benzene, toluene, ethylbenzene, and xylenes, Fig. 5), are soluble enough to contaminate groundwater. Although with the advent of oxygenated gasolines, it is expected that these oxygenates (ethanol, methanol, MTBE (methyl-*tert*-butyl ether) etc) will also be found in groundwater. In the past, remediation of such situations has usually used pump-and-treat methodologies. These methods are slow and may leave reservoirs of contaminants in pockets that are poorly connected to the main water body. Of course the contaminant is biodegradable, and some biodegradation is probably already occurring when the contamination is discovered. The cheapest approach to remediation is, thus, to allow this intrinsic process to continue. Evidence that it is indeed occurring can be found in the selective disappearance of the most biodegradable compounds in the contaminant mixture, and the



Benzene Toluene Ethylbenzene

o-Xylene *m*-Xylene *p*-Xylene

Fig. 5. The components of BTEX.

concomitant disappearance of electron acceptors from the groundwater. Thus oxygen is depleted as the preferential terminal electron acceptor for metabolism, followed by nitrate, ferric iron, sulfate, and finally CO_2 for methanogenesis (16).

Intrinsic bioremediation is becoming an acceptable option in locations where the contaminated groundwater poses little threat to environmental health. Nevertheless, although intrinsic bioremediation is appealingly simple, it may not be the lowest cost option if there are extensive monitoring and documentation costs involved for several years. In such cases it may well be more cost effective to optimize conditions for biodegradation.

One approach is to optimize the levels of electron acceptors. Oxygen can be pumped in as the pure gas, or as air, although this is relatively energy intensive since oxygen is so poorly soluble. Hydrogen peroxide has been used in some situations, but there have been problems with biomass plugging near the injection wells. Slow release formulations of inorganic peroxides, such as magnesium peroxide, have recently been used with success (17). Nitrate may be added, although there are sometimes regulatory limitations on the amount of this material that may be added to groundwater (18). Ferric iron availability may be manipulated by adding ligands (19).

If there are significant amounts of both volatile and nonvolatile contaminants, remediation may be achieved by a combination of liquid and vapor extraction of the former, and bioremediation of the latter. This combination has been termed "bioslurping", where the act of pumping out the liquid contaminant phase draws in air at other wells to stimulate aerobic degradation (20). Such bioremediation requires that there be enough nutrients to allow microbial growth, and fertilizer nutrients are frequently added at the air injection wells. Bioslurping has had a number of well-documented successes.

The majority of remediation operations include stopping the source of the contamination, but in some cases this is impossible, either because of the location of the spill, or because it is over a large area, and not a point source. In these situations it may be possible to intercept the flow of contaminated groundwater off-site, and ensure that no contamination passes. The simplest intervention is a line of wells for pump and treat, but including a biological component may be more cost effective. This can range from the installation of a sparge line for aerating the contaminated plume, to installing some form of semicontained bioreactor where nutrients can be applied with some modicum of control. Often these designs are combined with barriers to ensure that all the contaminated plume passes through the reactive zone. These designs have a variety of names, including biowall, trench biosparge, funnel and gate, bubble curtain, sparge curtain and engineered trenches and gates. Both aerobic and anaerobic designs have been successfully installed.

Where there are large volumes of contaminated water under a small site, it is sometimes most convenient to treat the contaminant in a biological reactor at the surface. Considerable research has gone into reactor optimization for different situations and a variety of stirred reactors, fluidized-bed reactors, and trickling filters have been developed. Such reactors are usually much more efficient than *in situ* treatments, although correspondingly more expensive.

Of course the presence of a liquid phase of hydrocarbon in a soil gives rise to vapor contamination in the vadose zone above the water table. This can be treated by vacuum extraction, and the passage of the exhaust gases through a biofilter (see above) can be a cheap and effective way of destroying the contaminant permanently.

Soil. Hydrocarbon contamination of soils runs the gamut from crude oils at production well and pipeline spills, to the full slate of refined products at refineries, distribution centers, service stations and accident sites. Significant hydrocarbon contamination is also often found at manufactured gas plants, now mainly abandoned, wood treatment facilities, railroad rights of way and terminals, and various military bases. Sometimes the contamination is the result of leaking underground storage tanks and pipelines, leading to subsurface contamination, but surface spills also occur. Physical removal of gross contamination is an obvious first step at all locations, and bioremediation is an appealing option for remediating residual contamination in many of these sites.

Spills from production facilities and pipelines often involve both oil and brine, since most oil reservoirs float on top of concentrated brines, and both are produced in later stages of production. The brine is typically separated from the oil and re-injected into the reservoir, but some is retained in many production pipelines. The environmental impact of spilled brine can be quite deleterious. Not only is salt toxic to most plants, and can inhibit many soil bacteria, but it also can have a major effect on the soil structure by altering the physical properties of clays. Successful bioremediation strategies must therefore include remediating the brine. In wet regions the salt is eventually diluted by rainfall, but in arid regions, and to speed the process in wetter regions, gypsum is often added to restore soil porosity.

Many hydrocarbons bind quite tightly to soil components, and are thereby less available to microbial degradation. The kinetics of binding seem to be complex, and the process of “aging” is only poorly understood. Nevertheless, it seems clear that hydrocarbons that have been in contact with soil for a long time are not as available for biodegradation as fresh spills. Several groups of researchers have suggested the addition of surfactants to overcome this limitation, but this approach is not yet widely used. A significant potential concern is that the surfactant will be degraded in preference to the contaminant of concern.

Intrinsic biodegradation occurs, but it usually only removes the lightest refined products, such as gasoline, diesel and jet fuel. Active intervention is typically required. Usually the least expensive approach is *in situ* remediation, typically with the addition of nutrients, and the attempted optimization of moisture and oxygen by tilling. Various approaches to applying fertilizers have been tried, including both standard and slow-release nutrients, oleophilic nutrients and solutions of liquid fertilizers. Oxygen is a likely limiting nutrient in many cases, and soil tilling is widely practiced. This *in situ* bioremediation of hydrocarbon-contaminated soils is akin to the old practice of “land-farming”, wherein sludges and other refinery wastes were deliberately spread onto soil and tilled and fertilized to stimulate biodegradation. Although this practice is now discontinued in the United States, it was quite widely used.

Deeper contamination may be remedied with bioventing, where air is injected through some wells, and extracted through others to both strip volatiles and provide oxygen to indigenous organisms. Fertilizer nutrients may also be added. This is usually only a viable option with lighter refined products.

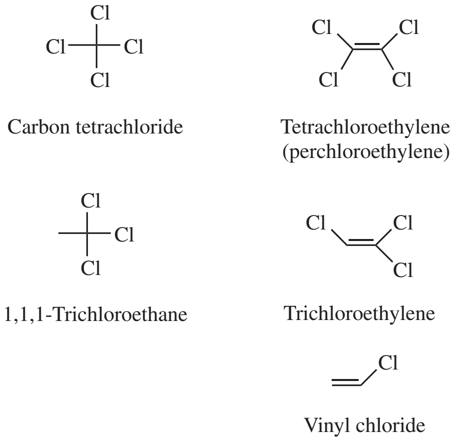
A recent suggestion has been to use plants to stimulate the microbial degradation of the hydrocarbon (hydrocarbon phytoremediation). This has yet to receive clear experimental verification, but the plants are proposed to help deliver air to the soil microbes, and to stimulate microbial growth in the rhizosphere by the release of nutrients from the roots. The esthetic appeal of an active phytoremediation project can be very great.

When soil contamination extends to some depth it may be preferable to excavate the contaminated soil and put it into “biopiles” where oxygen, nutrient and moisture levels are more easily controlled. Biopiles can also be kept warm during winter months, increasing the amount of time available for biodegradation in colder climates. Since the soil is well mixed during the construction of the pile, there is an opportunity to add selected microbial and fungal strains in an additional attempt to maximize biodegradation.

Composting by the addition of readily degradable bulking agents is also a useful option for relatively small volumes of excavated contaminated soil. Since efficient composting invariably involves self-heating as biodegradation proceeds, this also offers an option for extending the bioremediation season into the winter months in cold climates. A potential drawback of composting is that it usually increases the volume of contaminated material, but if fully successful the finished compost can be returned to the site as a positive contribution to soil quality.

Slurry bioreactors offer the most aggressive approach to maximizing contact between the contaminated soil and the degrading organisms. Both lagoons and reactor vessels have been used, but the former are often not optimally designed for all the soil to be partially suspended by the mixing impellers. Contained reactor designs include mixing tank, airlift, and fluidized-bed aeration. A major advantage of contained slurry bioreactors is the potential ability to optimize nutrients, aeration and degradative inocula as fresh soil is added, and the control of waste materials, including gases continually. Slurry bioreactors are usually the most expensive bioremediation option because of the large power requirements, but under some conditions this cost is offset by the rapid biodegradation that can occur.

In all these cases it is important to bear in mind that although the majority of hydrocarbons are readily biodegraded, some, such as the steranes and hopanes, are very resistant to microbial attack. Estimates of oil biodegradation range from 60–95% for different crude oils, so fresh spills of crude oils are readily treated by bioremediation (21). Refined products, such as gasoline, diesel, jet fuels, and heating oils are usually more biodegradable than typical whole oils, but the various heavy fractions of crude oils, such as the asphalts, are far less biodegradable, and are not such attractive targets for bioremediation. Some crude oils have already been extensively biodegraded in their reservoirs, and these are also poor targets for bioremediation. An example is Orimulsion, a heavy oil in water emulsion (70% bitumen) stabilized by low levels of surfactants, used as a fuel for electricity generation. Similarly, old spills may have already undergone significant biodegradation and the residue may be relatively biologically inert. It is thus important to run laboratory studies to ensure that the contaminant is sufficiently biodegradable that clean-up targets can be met.



Carbon tetrachloride

Tetrachloroethylene (perchloroethylene)

1,1,1-Trichloroethane

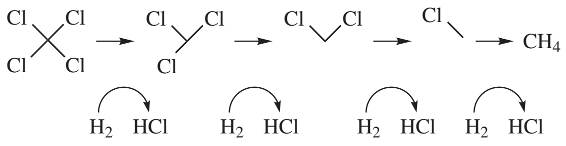
Trichloroethylene

Vinyl chloride

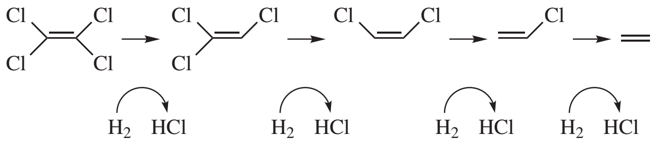
Fig. 6. Some representative halogenated solvents.

4.2. Halogenated Organic Solvents. *Constituents.* Halogenated organic solvents are widely used in metal processing, electronics, dry cleaning and paint, paper and textile manufacturing, and some representative examples are shown in Figure 6. These solvents have been used for more than fifty years, and unfortunately they are fairly widespread contaminants. Unlike the hydrocarbons, which usually float on water, the halogenated solvents typically have specific gravities greater than 1, and they generally sink to the bottom of any groundwater, and float on the bedrock. For this reason they are sometimes known as DNAPLs for dense nonaqueous phase liquids.

Biodegradation. Halogenated solvents are degraded under aerobic and anaerobic conditions. The anaerobic process is typically a reductive dechlorination that progressively removes one halide at a time (Fig. 7). For example, under methanogenic conditions, carbon tetrachloride is sequentially dechlorinated to chloroform, dichloromethane, methyl chloride and methane, while trichloroethylene



Carbon tetrachloride



Tetrachloroethylene

Fig. 7. Reductive dechlorination of carbon tetrachloride and tetrachloroethylene.

is sequentially reduced to ethylene. Some of these compounds are also dehalogenated under sulfate-reducing conditions, and under denitrifying conditions there are reports that the final product can be CO_2 (22). Chloromethane and dichloromethane have been shown to be the sole carbon source for several anaerobic organisms (23), and it seems there is much to be learned about the microbial diversity of anaerobic microorganisms capable of dechlorinating solvents.

The simplest chlorinated alkanes, alkenes, and alcohols (eg, chloromethane, dichloromethane, chloroethane, 1,2-dichloroethane, vinyl chloride, and 2-chloroethanol) serve as substrates for aerobic growth for some bacteria, but the majority of halogenated solvents cannot support growth (24). Nevertheless these compounds are mineralized under aerobic conditions, albeit with no apparent benefit to the degrading organism. Indeed, the oxidation appears to be fortuitous and it occurs during the metabolism of a growth substrate. The phenomenon is therefore known as co-metabolism or co-oxidation. Numerous bacteria are able to catalyze the oxidation of trichloroethylene; some use monooxygenases (for example methane and ammonia oxidizing species); others contain dioxygenases (eg, some toluene oxidizing species). The difference between these two classes of enzymes is the fate of the two atoms of molecular oxygen. Monooxygenases insert one oxygen atom into their substrate, and reduce the other to water. Dioxygenases insert both atoms into their substrate. The effect of these two types of enzyme is illustrated in Figure 8. In either case, biodegradation proceeds to complete mineralization (25).

The biodegradation of trichloroethylene is the most studied since this is probably the most widespread halogenated solvent contaminant. Several substrates drive trichlorethylene co-oxidation, including methane, propane, propylene, toluene, isopropylbenzene, and ammonia (25). The enzymes that metabolize these substrates have subtly different selectivities with regard to the halogenated solvents, and to date none are capable of co-oxidizing carbon tetrachloride or tetrachloroethylene. Complete mineralization of these compounds can, however, be achieved by sequential anaerobic and aerobic process.

Bioremediation. Air. Biofilters are an effective way of dealing with air from industrial processes that use halogenated solvents such chloromethane, dichloromethane, chloroethane, 1,2-dichloroethane and vinyl chloride, that support aerobic growth (26). Both compost-based dry systems and trickling filter wet systems are in use. Similar filters could be incorporated into pump-and-treat operations.

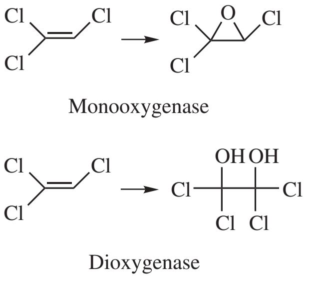


Fig. 8. Aerobic activation of tetrachloroethylene.

Groundwater and Soil. Halogenated solvents have contaminated soils and groundwater throughout the industrialized world, and remediation has a high priority. Since the solvents are so dense, they are typically found on the bedrock underlying aquifers. Pumping out the liquid phase is an obvious first step if the contaminant is likely to be mobile, but *in situ* bioremediation is a promising option. Thus, the U.S. Department of Energy is investigating the use of anaerobic *in situ* degradation of carbon tetrachloride in an aquifer some 76 m below the Hanford, Washington site, with nitrate as electron acceptor, and acetate as electron donor (22).

Trichloroethylene is the most frequent target of remediation, and as discussed above, this is only metabolized cometabolically. Remediation operations thus incorporate the addition of the cometabolized substrate. Methane was used successfully at the U.S. Department of Energy site at Savannah River, near Aiken, South Carolina (27) which had both an air-stripping and a biological component. Horizontal wells were used to pump methane and air below the contaminant, while an upper horizontal well in the vadose zone was used to withdraw these gases through the contaminated zone. Optimum biodegradation performance seemed to come from alternate injection of air and methane in air, and the inclusion of nitrous oxide and triethylphosphate, both gases, to give a C:N:P ratio of 100:10:1.

Plants may have a role to play in enhancing microbial biodegradation of halogenated solvents, for it has recently been shown that mineralization of radiolabelled trichlorothylene is substantially greater in vegetated rather than unvegetated soils (28), indicating that the rhizosphere provides a favorable environment for microbial degradation of organic compounds.

Methane has also been used in aerobic bioreactors that are part of a pump-and-treat operation, and toluene and phenol have also been used as co-substrates at the pilot scale (29). Anaerobic reactors have also been developed for treating trichloroethylene. For example, Wu and co-workers (30) have developed a successful upflow anaerobic methanogenic bioreactor that converts trichloroethylene and several other halogenated compounds to ethylene.

Groundwater contaminated with other halogenated solvents can also be treated in aboveground reactors. Aerobic reactors are useful for those compounds that can support growth. For example, a membrane reactor has been designed for treating 1,2-dichloroethane (31), and bubble columns and packed bed reactors have been developed for the aerobic degradation of 2-chloroethanol (32). As mentioned above, sequential anaerobic and aerobic reactors are capable of mineralizing tetrachloroethylene (33).

4.3. Halogenated Organic Compounds. *Constituents.* Complex halogenated organic compounds have been widely used in commerce in the last fifty years. A few representative examples are shown in Figure 9; pentachlorophenol has been widely used as a wood preservative, and also for termite control. (2,4-Dichlorophenoxy)acetate (2,4-D) is widely used as a broad-leaf herbicide, DDT was widely used as an insecticide, and hexachlorophene has been widely used as a germicide. Polychlorinated biphenyls (PCBs) were sold with varying levels of chlorination for a range of purposes. They ranged from light oily fluids (with two, three, or four chlorines) to viscous oils (five chlorines) to greases and waxes (six or more chlorines), and their names indicated the level of chlorination.

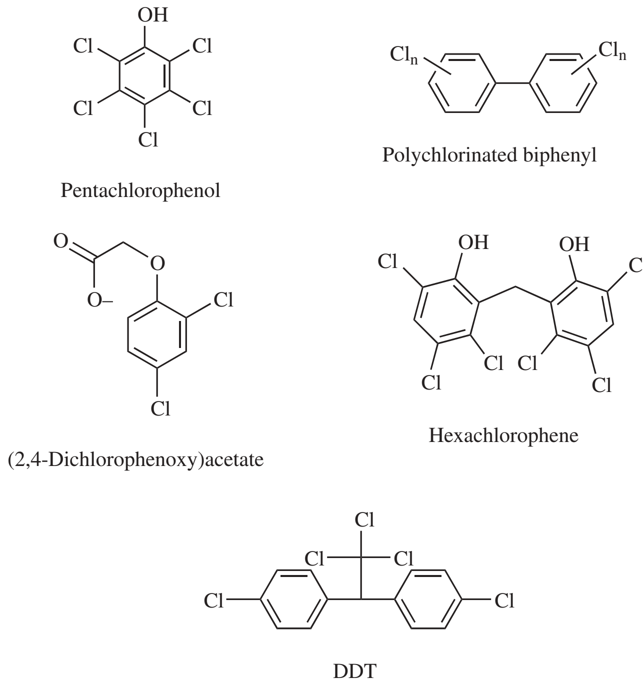


Fig. 9. Representative halogenated organic contaminants.

Thus Aroclor 1242 (trademark of Monsanto, U.S.), Clophen A30 (trademark of Farbenfabriken Bayer AG, Germany) and Kanechlor 300 (trademark of Kanegafuchi Chemical Industries, Japan) all contained 42% chlorine by weight, and an average of three chlorines per biphenyl. An important property shared by all these compounds is their relative resistance to biodegradation, so at first glance they may not seem a good target for bioremediation. Indeed complex halogenated organic compounds were widely thought to be almost exclusively anthropogenic in origin, so that there would have been little time for biodegradation pathways to evolve. This view is being corrected, for in fact a variety of organisms, particularly marine algae and some fungi, produce significant quantities of these compounds (34). There is, thus, good reason to expect that halogenated-organic degrading organisms will be found in the biosphere, and this has been borne out in practice.

Biodegradation. An important characteristic of degradation is the cleavage of carbon–chlorine bonds, and the enzymes that catalyze these reactions, the dehalogenases, are being characterized (35). The reductive dechlorination seen with carbon tetrachloride and tetrachloroethylene (see Fig. 7) seems to be a general phenomenon, and even compounds as persistent as DDT and the polychlorinated biphenyls are reductively dechlorinated under some conditions, particularly under methanogenic conditions. Some compounds, such as pentachlorophenol, can be completely mineralized under anaerobic conditions, but the more recalcitrant ones require aerobic degradation after reductive dehalogenation.

Pentachlorophenol can be mineralized aerobically and anaerobically, and both processes have been exploited for bioremediation. Under methanogenic conditions a reductive dehalogenation, analogous to that seen with halogenated solvents, eventually generates phenol, although some of the intermediate congeners are quite recalcitrant (36). The phenol is further mineralized to methane and carbon dioxide, and sulfate-reducing bacteria may be involved (37).

Several bacterial isolates are able to grow aerobically using pentachlorophenol as sole source of carbon and energy, but many grow rather better when supplemented with a more nutritions substrate. Unfortunately, in many cases it seems that the more vigorous growth with these substrates does not enhance the biodegradation of pentachlorophenol (38). The initial step in pentachlorophenol biodegradation in one *Flavobacterium* species is an NADPH-dependent oxygenolytic dechlorination, where the *para*-chloro group is replaced by a hydroxyl to generate tetrachlorohydroquinone. Other species seem to produce the same metabolite by a hydrolytic process (38). Fungal degradation of pentachlorophenol, apparently using the lignolytic apparatus, has also been reported (39).

(2,4-Dichlorophenoxy)acetate (2,4-D) has been one of the world's most popular herbicides. Although it is somewhat resistant to biodegradation, it is biodegraded by several bacterial isolates. It is a general truism that the more halogens on a molecule, the slower its biodegradation, and this is borne out with the related herbicide 2,4,5-T ((2,4,5-trichlorophenoxy)acetate). Nevertheless, bacterial degradation has been seen under both aerobic and anaerobic conditions, the latter involving reductive dechlorination via 2,4-D. Aerobic degradation removes acetate from (2,4-dichlorophenoxy)acetate to yield 2,4-dichlorophenol, which is subsequently hydroxylated to 3,5-dichlorocatechol, followed by ring cleavage and complete mineralization (40). Genetic engineering has been used to construct strains that are particularly adept at consuming these compounds, but whether these will overcome the regulatory hurdles to allow their use outside the laboratory remains to be seen.

DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is a remarkably resistant molecule, which explains both its efficacy as an insecticide, and its accumulation at the top of the food-chain. Nevertheless, there are indications that it can be biodegraded, both anaerobically with initial dechlorination and aerobically with initial ring hydroxylation (40). DDT and its partially degraded congeners are very hydrophobic, and biodegradation seems to be stimulated by adding surfactants. White-rot fungi also degrade DDT under lignolytic conditions, although there is little mineralization to CO_2 (5).

The polychlorinated biphenyls are quite recalcitrant. Some lightly chlorinated biphenyls are readily mineralized under aerobic conditions (41), and indeed the structure of an enzyme that catalyzes the key ring-cleavage oxygenation has recently been determined by x-ray crystallography (42). More chlorinated congeners are resistant to aerobic degradation, but they are reductively dechlorinated under anaerobic conditions. Complete degradation of the commercial mixtures, thus, generally requires an anaerobic process followed by an aerobic one. A major issue for the oxidative process is that biphenyl seems to be required for significant expression of the biodegradative system (41); the chlorinated compounds do not induce the enzymes that would degrade them. The biochemistry of the biodegradative process is only beginning to be unraveled, but already there are suggestions that there is considerable diversity in the enzyme systems able to degrade these compounds. There is a lot of effort aimed at engineering organisms to degrade polychlorinated biphenyls, and in finding ways to make these very hydrophobic compounds more bioavailable by the use of chemical oxidants, such as Fenton's reagent (43), or surfactants.

Bioremediation. Soil. Pentachlorophenol has been the target of bioremediation at a number of wood-treatment facilities, and good success has been achieved in several applications. It is rarely the sole contaminant, and is often present with polynuclear hydrocarbons from coal creosote. *In situ* degradation has been stimulated by bioventing, where air is injected through some wells, and extracted through others to both strip volatiles and provide oxygen to indigenous organisms (44). Just as with the halogenated solvents, it seems that plants stimulate microbial degradation of pentachlorophenol in the rhizosphere (45).

The kinetics of such *in situ* degradation are rather slow, however, and more active bioremediation is usually attempted. For example, contaminated soil at the Champion Superfund site in Libby, Montana, was placed into 1-acre land treatment units in 6-in. layers, and irrigated, tilled, and fertilized. Under these conditions, the half-lives of pentachlorophenol, pyrene, and several other polynuclear aromatic hydrocarbons, initially present at around 100–200 ppm, were on the order of 40 days (46). This success relied on the indigenous microbial populations in the soil, but many groups are focusing on the addition of organisms (eg, 47), perhaps immobilized on some sort of carrier. Composting, and bioremediation focusing on the use of white-rot fungi, has also met with success at the pilot scale. Others have used fed-batch or fluidized-bed bioreactors to stimulate the biodegration of pentachlorophenol. This allows significant optimization of the process and increases in rates of degradation by tenfold (48).

A major concern when remediating wood-treatment sites is that pentachlorophenol was often used in combination with metal salts, and these compounds, such as chromated copper–arsenate, are potent inhibitors of at least some pentachlorophenol degrading organisms (49). Sites with significant levels of such inorganics may not be suitable candidates for bioremediation.

The phenoxy-herbicide, 2,4-D, has been successfully bioremediated in a soil contaminated with such a high level of the compound (710 ppm) that it was toxic to microorganisms (50). There were essentially no indigenous bacteria in the soil. Success relied on washing a significant fraction of the contaminant off the soil and adding bacteria enriched from a less contaminated site. Success was achieved in remediating both soil washwater and soil in a bioslurry reactor (50). 2,4-D is also effectively degraded in composting, with about half being completely mineralized, and the other half becoming incorporated in a nonextractable form in the residual soil organic matter (51).

The bioremediation of polychlorinated biphenyls in soils in receiving significant attention because these compounds are quite widely distributed in the environment, either from leaking electrical transformers or sometimes because they were applied as part of road maintenance. In the latter case, the contamination usually includes petroleum hydrocarbons, and unfortunately it seems that the two contaminants inhibit the degradation of each other. Nevertheless, cultures are being found that can degrade both polychlorinated biphenyls and petroleum hydrocarbons. There is also interest in the role of rhizosphere organisms in polychlorinated biphenyl degradation, particularly since some plants exude phenolic compounds into the rhizosphere that can stimulate the aerobic degradation of the less chlorinated biphenyls.

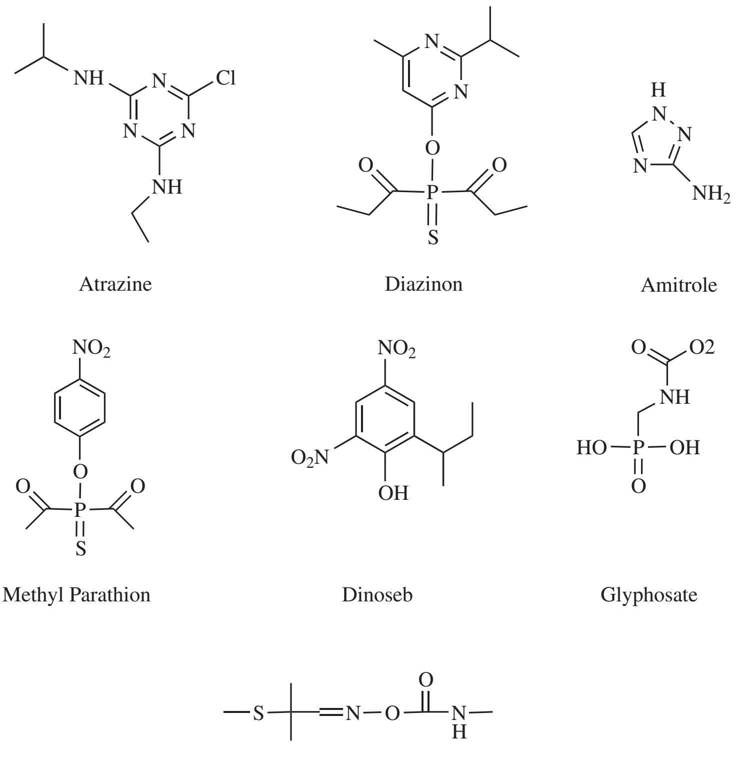
Groundwater. A successful groundwater bioremediation of pentachlorophenol is being carried out at the Libby Superfund site described above. A shallow aquifer is present at 5.5 to 21 m below the surface, and a contaminant plume is nearly 1.6 km in length. Nutrients and hydrogen peroxide were added at the source area and approximately half way along the plume, and pentachlorophenol concentrations decreased from 420 ppm to 3 ppm where oxygen concentrations were successfully raised. A membrane oxygen dissolution system has now been installed to replace the hydrogen peroxide additions, and costs have been substantially lowered without an apparent decrease in remediation performance (52).

Pentachlorophenol is readily degraded in biofilm reactors (53), so bioremediation is a promising option for the treatment of contaminated groundwater brought to the surface as part of a pump-and-treat operation.

River and Pond Sediments. Much of the work on polychlorinated biphenyls has focused on the remediation of aquatic sediments, particularly from rivers, estuaries, and ponds. As noted above, a few of the most lightly chlorinated compounds are mineralized under aerobic conditions, but the more chlorinated species seem completely resistant to aerobic degradation, even by white rot fungi (54). On the other hand, there is extensive dechlorination of highly chlorinated forms under anaerobic conditions, particularly methanogenic conditions. Bioremediation thus requires anaerobic and aerobic regimes. Intrinsic biodegradation of polychlorinated biphenyls can be recognized by the changing “fingerprint” of the individual isomers as biodegradation proceeds (41,55). The anaerobic dechlorination of the most recalcitrant congeners can apparently be primed by adding a readily dehalogenated congener, such as 2,5,3′,4′-tetrachlorobiphenyl (56), but whether this is a realistic approach for *in situ* bioremediation remains to be seen. Harkness and co-workers (57) have successfully stimulated aerobic biodegradation in large caissons in the Hudson River by adding inorganic nutrients, biphenyl, and hydrogen peroxide, but found that repeated addition of a polychlorinated-biphenyl degrading bacterium (*Alcaligenes eutrophus* H850) had no beneficial effect. Essentially no biodegradation occurred in the stirred control caissons, but losses on the order of 40% were seen in the caissons that received nutrients and peroxide, regardless of whether the stirring was aggressive or rather gentle. Whether this approach can be scaled-up for large-scale use, with a net environmental benefit, remains to be seen.

4.4. Nonchlorinated Pesticides and Herbicides.

Constituents. A vast number of compounds are used as herbicides, pesticides, fungicides, etc, and a few are shown in Figure 10. In order to be effective they must have their effect before they are degraded in the environment, but on the other hand they must not be so resistant to degradation that they accumulate where they are used, or accumulate in food chains. It is unusual for these compounds to become contaminants where they are applied correctly, but manufacturing facilities, storage depots and rural airfields where crop-dusters are based have had spills that can lead to long lasting contamination. Bioremediation is a promising



Atrazine

Diazinon

Amitrole

Methyl Parathion

Dinoseb

Glyphosate

Aldicarb

Fig. 10. Examples of herbicides and pesticides amenable to bioremediation.

technology to remediate such sites. There are also some locations were groundwater has become contaminated by these chemicals, and again bioremediation may be a cost-effective remediation strategy.

Biodegradation. The vast majority of pesticides, herbicides, fungicides, and insecticides in use today are biodegradable, although the intrinsic biodegradability of individual compounds is one of the variables used in deciding which compound to use for which task. Some herbicides are acutely toxic to plants that absorb them, but are so readily degraded in soil that seeds can be planted at the same time as the herbicide application. Other herbicides are known to be effective at preventing plant growth for many months, and are used in situations where this is the desired goal. Very few degradation pathways of these compounds have been worked out in detail, but some generalizations can be made.

Compounds with organophosphate moieties, such as Diazinon, Methyl Parathion, Coumaphos and Glyphosate are usually hydrolyzed at the phosphorus atom (40,58). Indeed several *Flavobacterium* isolates are able to grow using parathion and diazinon as sole sources of carbon.

Triazines pose rather more of a problem, probably because the carbons are in an effectively oxidized state so that no metabolic energy is obtained by their metabolism. Very few pure cultures of microorganisms are able to degrade triazines such as Atrazine, although some *Pseudomonads* are able to use the compound as sole source of nitrogen in the presence of citrate or other simple carbon substrates. The initial reactions seem to be the removal of the ethyl or isopropyl substituents on the ring (41), followed by complete mineralization of the triazine ring.

Nitroaromatic compounds, such as Dinoseb, are degraded under aerobic and anaerobic conditions (59). The nitro group may be cleaved from the molecule as nitrite, or reduced to an amino group under either aerobic or anaerobic conditions. Alternatively, the ring may be the subject of reductive attack. Thus, while these molecules are sometimes quite long-lived in the environment, they can be completely mineralized under appropriate conditions (59). Recent work has isolated a *Clostridium bifermentans* able to anaerobically degrade dinoseb cometabolically in the presence of a fermentable substrate. The dinoseb was degraded to below detectable levels, although only a small fraction was actually mineralized to CO_2 (60).

Carbamates such as Aldicarb undergo degradation under both aerobic and anaerobic conditions. Indeed the oxidation of the sulfur moiety to the sulfoxide and sulfone is part of the activation of the compound to its most potent form. Subsequent aerobic metabolism can completely mineralize the compound, although this process is usually relatively slow so that it is an effective insecticide, acaricide and nematocide. Anaerobically these compounds are hydrolyzed, and then mineralized by methanogens (61).

Bioremediation. Groundwater. Atrazine dominated the world herbicide market in the 1980s, and contamination of groundwater has been reported in several locations in the U.S., Europe, and South Africa. There are several reports that once in groundwater it is very recalcitrant, suggesting that atrazine-degrading organisms are not widespread. Nevertheless, successful biodegradation has been achieved with indigenous organisms in laboratory mesocosms after a lag phase, and once activity was found, it remained (62). Interestingly the degradation was somewhat slowed by the addition of low concentrations of readily assimilate carbon, such as lactate, and it is not clear how biodegradation might be stimulated in the field. Nevertheless, it is clear that intrinsic remediation is likely to lead to the disappearance of atrazine from groundwaters once atrazine-utilizers have become abundant, and perhaps inoculation with atrazine-metabolizing species will be effective.

If more active treatment is required, such as pump-and-treat, it is possible that biological reactors will be a cost-effective replacement for activated carbon filters (63).

Marsh and Pond Sediments. Herbicides and pesticides are detectable in marsh and pond sediments, but intrinsic biodegradation is usually found to be occurring. Little work has yet been presented where the biodegradation of these compounds has been successfully stimulated by a bioremediation approach.

Soil. Herbicides and pesticides are of course metabolized in the soil to which they are applied, and there are many reports of isolating degrading organisms from such sites. Degradation activities are typically much higher at sites that have seen product application, indicating that natural enrichment processes occur. Much current effort is aimed at assessing the diversity of degradative pathways, and in many cases it seems that several different natural metabolic pathways can degrade individual pollutants. Little work has yet been presented where the biodegradation of these compounds has been successfully stimulated by a bioremediation approach, but inoculation with active organisms may be a promising approach (64,65).

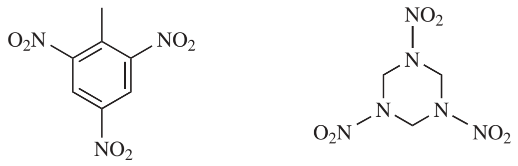
It is a general observation that herbicide degradation occurs more readily in cultivated than fallow soil, suggesting that rhizosphere organisms are effective herbicide degraders. Whether this can be effectively exploited in a phytoremediation strategy remains to be seen.

4.5. Military Chemicals.

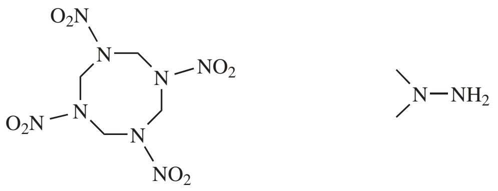
Constituents. The military use a range of chemicals as explosives and propellants, which are sometimes termed "energetic molecules". Generally speaking, modern explosives are cyclic, often heterocyclic, composed of carbon, nitrogen and oxygen. Perhaps the most well known is 2,4,6-trinitrotoluene (Fig. 11), but RDX (Royal Demolition eXplosive; hexahydro-1,3,5-trinitro-1,2,3-triazine) and HMX (High Melting eXplosive; octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), are even more powerful. *N*,*N*-Dimethylhydrazine is used as a solid rocket fuel. These compounds are sometimes present at quite high levels in soils and groundwater on military bases and production sites. One quite infamous problem at the latter is "pink water", a relatively undefined mixture of photodegradation products of TNT. Bioremediation is a promising new technology for treating sites contaminated with such compounds.

Bioremediation may also be an appropriate tool for dealing with chemical agents such as the mustards and organophosphate neurotoxins (66), but little work on actual bioremediation has been published.

Biodegradation. Natural nitrosubstituted organic compounds are quite unusual, and it was once thought that their degradation was principally by abiotic processes. As shown above in the case of Dinoseb, however, nitrosubstituted compounds are subject to a variety of degradative processes. The biodegradation of TNT is well established (67). Under anaerobic conditions it is readily reduced to the corresponding aromatic amines and subsequently deaminated to toluene. As shown in the section on hydrocarbons, the latter can be mineralized under



TNT RDX



HMX *N*,*N*-Dimethylhydrazine

Fig. 11. Military explosives and a rocket propellant.

anaerobic conditions, leading to the potentially complete mineralization of TNT in the absence of oxygen.

Under aerobic conditions TNT can be mineralized by a range of bacteria and fungi, often co-metabolically with the degradation of a more degradable substrate. There is even evidence that some plants are able to deaminate TNT reductively. However, there is also ample evidence that under some conditions, the TNT is converted to insoluble large molecular weight compounds. This probably occurs by addition reactions to soil components, such as humic and fulvic acids, or cellular material in the case of plants. Lignolytic fungi are also yielding promising results for the degradation of both TNT and "pink water", particularly if the latter is pretreated with uv irradiation.

RDX and HMX are rather more recalcitrant, especially under aerobic conditions, but there are promising indications that biodegradation can occur under some conditions, especially composting (67). Several strains of bacteria able to use RDX (and Triazine) as a sole source of nitrogen for growth have recently been isolated, and this is an area where rapid progress is being made.

Little work has been reported on the biodegradation of dimethylhydrazine, but it may become an important target for remediation at some sites (68).

Bioremediation. Groundwater. Nitrotoluenes have been detected in groundwater in some areas, and intrinsic remediation may be occurring at some sites by anaerobic degradation. Research into whether this can be stimulated with a net environmental benefit is in its very earliest stages, and no clear evidence for success has been presented.

A commercial technology (69), the SABRE process, treats contaminated water and soil in a two-stage process by adding a readily degradable carbon and an inoculum of anaerobic bacteria able to degrade the contaminant. An initial aerobic fermentation removes oxygen so that the subsequent reduction of the contaminant is not accompanied by oxidative polymerization.

Soil. Composting of soils contaminated by high explosives is being carried out at the Umatilla Army Depot near Hermiston, Oregon (70). Soil from munitions washout lagoons is being treated indoors in compost rows of 2,000 m^3, and the estimated cost is less than one-third the estimated cost of incineration. If this is successful, there are 30 similar sites on the National Priority List that could be treated in a similar way.

4.6. Other Organic Compounds. The majority of organic compounds in commerce are biodegradable, so bioremediation is a potential option for cleaning up after industrial and transportation accidents. For example, Ref. 71 reports the successful bioremediation of 23,000 liters of vinyl acetate spilled from a railroad tank car in Albany, New York, at a cost approximately half that of excavation and disposal of the 1,100 m^3 of contaminated soil. They also report that *in situ* biological treatment has been used to remediate spills of other organics, including acrylonitrile, styrene, 2-butoxyethanol, and ethacrylate, at other sites where there had been railroad accidents. Bioremediation is thus already an important tool in remediating accidental spills of organic compounds.

Bioremediation is also an option when spills of such compounds contaminate groundwater. For example, bioremediation seems a feasible treatment for aquifers contaminated with alkylpyridines (72) and phenol (73).

5. Inorganic Contaminants

5.1. Nitrogen Compounds. *Constituents.* Nitrogen-containing compounds are of concern for several reasons. Nitrate levels are regulated in groundwater because of concerns for human and animal health. Ammonia is regulated in streams and effluents as a potential fish toxicant, and any nitrogenous contaminant is a potential problem in water because of its stimulatory effect on the growth of algae. Other nitrogenous contaminants include cyanides in mine waters. Fortunately, all are amenable to biological treatment.

5.2. Biodegradation. The biological mineralization of fixed nitrogen is well studied; ammonia is oxidized to nitrite, and nitrite to nitrate, by autotrophic bacteria, and nitrate is reduced to nitrogen by anaerobic bacteria. Urea in sewage and industrial wastes is readily hydrolyzed to ammonia and CO_2 by many bacteria, and cyanides and cyanates are used as sole sources of carbon and nitrogen by some organisms. Wastewater treatment facilities utilize these organisms in assuring that municipal and industrial effluents meet strict water quality standards, but this biological process is outside the scope of this article (see WATER, INDUSTRIAL WATER TREATMENT; WATER, MUNICIPAL WATER TREATMENT). On the other hand, ammonia and nitrate are essential nutrients for plant and bacterial growth, so one option is to use these organisms to take up and use the contaminants.

Bioremediation. Surface Water. One example of exploiting biology to handle excess nitrogen in a surface water is the case of the Venice Lagoon in Italy. About a million tons of sea lettuce (*Ulva*) grows in the lagoon annually because of the high levels of nitrogenous nutrients in this relatively landlocked bay. This material is harvested, composted and sold as a low-cost remediation of this problem (74). In areas where water quality is sufficiently high, growing even more valuable crops, such as nori (*Porphyra*) may be an attractive bioremediation option.

A more constrained opportunity for nitrate bioremediation arose at the US-DoE Weldon Spring Site near St. Louis, Missouri. This site had been a uranium and thorium processing facility, and treatment of the metal had involved nitric acid. The wastestream, known as raffinate, was discharged to surface inpoundments and neutralized with lime to precipitate the metals. Two pits had nitrate levels that required treatment before discharge, but heavy rains in 1993 threatened to cause the pits to overflow. Bioremediation by the addition of calcium acetate as a carbon source successfully treated more than 19 million liters of water at a reasonable cost (75).

Groundwater. One approach to minimizing the environmental impact of excess nitrogen in groundwater migrating into rivers and aquifers is to intercept the water with rapidly growing trees, such as poplars, that will use the contaminant as a fertilizer.

An alternative approach is to add a readily degradable substrate to the contaminated aquifer, in the absence of oxygen, to stimulate bacterial denitrification. Soluble substrates such as ethanol have been suggested, but a recent alternative suggestion is to provide vegetable oil, either down the well itself, or on a gravel trickling filter. Since the oil is insoluble in water, it becomes immobilized on the aquifer material, allowing denitrification as the water flows through (76).

5.3. Metals and Metalloids. A wide range of metallic and nonmetallic elements are present as contaminants at industrial and agricultural sites throughout the world, both in ground- and surface water, and in soils. They pose a quite different problem from that of organic contaminants, since they cannot be degraded so that they disappear. Some metal and metalloid elements have radically different bioavailabilities and toxicities depending on their redox state, so one option is stabilization by converting them to their least toxic form. This can be a very effective way of minimizing the environmental impact of a contaminant, but if the contaminant is not removed from the environment, there is always the possibility that natural processes, biological or abiological, may reverse the process. Removing the contaminants from water phases is relatively straightforward, and the wastewater treatment industry practices this on an enormous scale. Pump-and-treat systems that mimic waste water treatments are already being used for several contaminants, and less complex systems involving biological mats are a promising solution for less demanding situations.

Microbial leaching of metals from ores is a promising adjunct to more aggressive metal recovery technologies (77), but is generally achieved by oxidative processes that generate very acidic waters. It seems unlikely that similar approaches will be of much value in removing contaminant metals and metalloids from soils.

In the past, removing metal and metalloid contaminants from soil has been impossible, and site clean-up has meant excavation and disposal in a secure landfill. An exciting new approach to this problem is phytoextraction, where plants are used to extract contaminants from the soil and harvested.

Immobilization and Toxicity-Minimization. Adsorption. Biomass, often agricultural by-products, has been widely used as an adsorbent for metals and other contaminants in water (78), but this is outside the scope of this article.

Microbial Processes. Most elements display a range of solubilities and biological effects depending on their chemical form. For example, chromium, although it may be an essential micronutrient for many organisms, is known to be toxic at higher levels. Indeed chromium has a range of available redox states that differ significantly in their environmental impact. Cr(VI) is generally more soluble and more toxic than Cr(III), but a wide range of organisms, both bacteria and fungi, are able to reduce the former to the latter under both aerobic and anaerobic conditions. Thereby the environmental impact of the contaminant is reduced. Since Cr(III) precipitates as insoluble hydroxides under neutral and alkaline conditions, microbial reduction is a potential treatment for soils and waters (79).

Similarly selenium is a micronutrient that is toxic at higher levels. It also has quite different bioavailability in its different redox states, with the elemental form being the least biologically available. Many microorganisms, both bacteria and fungi, are able to reduce more oxidized species, especially selenite (Se(IV)) to the red elemental form, providing an appealing remediation option for this element. An alternative approach to remediating selenium contamination is to encourage methylation to volatile dimethylselenide (80). Although the microbiology of this process is not yet very well understood, it seems to be the result of degradation of the selenium-containing amino acid, selenomethionine.

Arsenic is another element with different bioavailability in its different redox states. Arsenic is not known to be an essential nutrient for eukaryotes, but arsenate (As(V)) and arsenite (As(III)) are toxic, with the latter being rather more so, at least to mammals. Nevertheless, some microorganisms grow at the expense of reducing arsenate to arsenite (81), while others are able to reduce these species to more reduced forms. In this case it is known that the element can be immobilized as an insoluble polymetallic sulfide by sulfate reducing bacteria, presumably adventitiously due to the production of hydrogen sulfide (82). Indeed many contaminant metal and metalloid ions can be immobilized as metal sulfides by sulfate reducing bacteria.

A rather more specific mechanism of microbial immobilization of metal ions is represented by the accumulation of uranium as an extracellular precipitate of hydrogen uranyl phosphate by a *Citrobacter* species (83). Staggering amounts of uranium can be precipitated; more than 900% of the bacterial dry weight! Recent work has shown that even elements that do not readily form insoluble phosphates, such as nickel and neptunium, may be incorporated into the uranyl phosphate crystallites (84). The precipitation is driven by the production of phosphate ions at the cell surface by an external phosphatase. Although the process requires the addition of a phosphate donor, such as glycerol-2-phosphate, it may be a valuable tool for cleaning water contaminated with radionuclides. An alternative mode of uranium precipitation is driven by sulfate-reducing bacteria such as *Desulfovibrio desulfuricans*, which reduce U(VI) to insoluble U(IV). When combined with bicarbonate extraction of contaminated soil, this may provide an effective treatment for removing uranium from contaminated soil (85).

Microbial processes can also detoxify mercury ions and organic compounds by reducing the mercury to the elemental form, which is volatile (86). This certainly reduces the environmental impact of compounds such as methylmercury, however, such a bioprocess would have to include a mercury capture system before it could be exploited on a large scale with public support.

Rhizofiltration. Rhizofiltration is the use of plant root systems to remove contaminating metal ions from water (87). The plants must have substantial root systems, and success has been reported with both hydroponically grown terrestrial plants, such as sunflowers (*Helianthus annuus*), and floating aquatic plants such as water hyacinth (*Eichhornia crassipes*), and water milfoil (*Myriophyllum spicatum*). A filamentous cyanobacterium, *Phormidium* sp., has also proven quite effective at removing metals from water. Understanding rhizofiltration at the molecular level is at an early stage, but it is already clear that several processes are involved. Adsorption of the contaminant to the root surface must occur first, and presumably happens in all plants, probably to polygalacturonic acids for cations, and positively charged polypeptides for anions. Some plants actively translocate the metal ions into their cells and some transport the metal to the leafy shoots. This can be exploited in phytoextraction of metal ions from soils (see below). This phenomenon may actually be undesirable in rhizofiltration, because if the metals are retained in the roots there will be a smaller volume of contaminated material to treat at the end of the water treatment. Perhaps of more importance for rhizofiltration is the process of root assisted precipitation, where metal ions are precipitated on the roots as insoluble inorganic complexes. For example, lead is precipitated as lead phosphates on roots of Indian Mustard

(*Brassica juncea*) to a remarkable level; up to 45% of dry weight (87). This phenomenon seems to require active exudation of precipitants from the roots, for it is dramatically reduced when dead roots are used.

Phytoextraction. The fact that certain plant species are able to survive on soils with such high levels of metal ions that the growth of most plants is inhibited has been known for centuries. However, it was not until this century that it became clear that some of these plants actually accumulated the potentially toxic metals and somehow resisted their toxic effects. These plants, known as hyperaccumulators, are exemplified by Alpine Pennycress, *Thlaspi caerulescens*, which is able to accumulate a wide range of metals, including cadmium, chromium, copper, lead, nickel, and zinc, more than several thousand fold in its roots, and somewhat less in its leaves (88). Unfortunately for a use in bioremediation, most hyperaccumulators are small, slow growing plants. Researchers have, therefore, turned to more rapidly growing plants which, while they might not be quite as effective as the hyperaccumulators on a weight basis, might be able to extract more metal in a growing season (88). Indian mustard, *Brassica juncea*, was found to be almost as effective as *T. caerulescens* and to grow much faster. This plant is now being used in field trials of phytoextraction.

Phytoextraction of metals from soils requires plants that have substantial root systems to maximize contact with the contaminated soil, and effective transport mechanisms to get the metal from the root to harvestable biomass. Merely accumulating the metal in or on the roots would be less desirable, because harvesting would likely increase the environmental impact of the contaminant by creating dust. It has been known for some time that metals such as cadmium are often stored in plant tissues as phytochelatin-complexes. Phytochelatins are small peptides rich in cysteines that chelate metals via the cysteinyl sulfur (89). Other elements are stored as metalphytate precipitates (88), and in both cases seem to be concentrated in the vacuolar sap. But how the metals are transported in the xylem sap from the root to the shoot is only beginning to be explored. Cadmium seems to be transported as a soluble salt of organic acids (90), at least in *B. juncea*, while nickel is thought to be transported as a soluble histidine complex in *Alyssum* species (91). Optimizing this process will be an important consideration for the commercial success of phytoremediation.

It will also be important to understand the rhizosphere ecology around the roots of metal accumulating plants fully. Maximizing the bioavailability of the contaminant metals in this zone may require the optimization of the microbial communities, or perhaps the addition of soil amendments. There are early indications that such intervention may be beneficial (88), but research in this area is at a very early stage.

Phytoremediation is also being developed for dealing with soils contaminated with high levels of selenium in California; again *B. juncea* seems to be particularly effective in accumulating the contaminant from soil, and all plants tested were more effective at removing selenate than selenite (92). This is an interesting contrast to bacterial systems, where selenite reduction is more commonly found than selenate reduction.

There have also been reports that some plants, including *B. juncea*, stimulate volatilization of dimethylselenide, although it seems that this is an indirect effect where the plant roots stimulate bacterial evolution of the gas (93). Presumably the selenium is eventually oxidized in the atmosphere and returned to the soil as rain. Since much of the world is marginally selenium deficient, such a process might have no deleterious environmental effects. A similar volatilization approach may eventually be used for mercury contamination. Ref. 94 describes the effective transfer of bacterial mercury reduction genes into a plant. In the future, this approach might offer an option for cleaning mercury-contaminated soils, albeit with some form of mercury capture technology.

Phytostabilization. In some cases it may be important to stabilize a contaminated site to minimize harmful environmental impacts. Barren soils are more prone to erosion, leaching, and dust formation than vegetated soils, so establishing plants as a ground cover may be a very beneficial treatment, even if the contaminant remains in place. In some cases, planting metal tolerant species is enough to establish a plant community, but in other situations, such as mine waste piles, it may be essential to restore fertility to the soil by adjusting the pH and adding organic composts. Where phytoextraction requires plants that actively transport metals to the above-ground biomass, this would be an undesirable phenomenon in phytostabilization programs because it would promote the movement of the contaminating metals into the biosphere.

It may also be possible to stimulate bioreductions in soils, such as Cr(VI) to Cr(III), more effectively by growing plants than by adding bacteria or nutrients to stimulate the microbial process.

Bioremediation. Water. Groundwater can be treated in anaerobic bioreactors that encourage the growth of sulfate reducing bacteria, where the metals are reduced to insoluble sulfides, and concentrated in the sludge. For example, such a system is in use to decontaminate a zinc smelter site in the Netherlands (95).

Bacterial remediation of selenium oxyanions in San Joaquin, California, drainage water is under active investigation (96, 97), but has not yet been commercialized. Agricultural drainage rich in selenium is also typically rich in nitrates, so bioremediation must also include conditions that stimulate denitrification (98). Phytoextraction of selenium is also being tested, but is not yet being used on a large scale.

Phytoremediation is also not yet being used commercially, but results at several field trial suggest that commercialization is not far away. Perhaps the biggest success to date is the successful rhizofiltration of radionuclides from a Department of Energy site at Ashtabula, Ohio, where uranium concentrations of 350 ppb were reduced to less than 5 ppb, well below groundwater standards, by Sunflower roots (99). Small field tests also suggest that rhizofiltration will be a remediation option for removing radionuclides, such as ^{137}Cs and ^{90}Sr, from contaminated lakes and ponds near the Chernobyl nuclear reactor in Ukraine.

Mine Drainage. Natural drainage water that come into contact with active and abandoned metal and coal mines can become seriously contaminated with a range of heavy-metal ions, and/or often become quite acidic, with a pH near 2. While underground, the water is typically anoxic, and any iron is present as soluble ferrous species. When this mixes with aerobic surface water, the iron precipitates as bright orange ferric hydroxide, and this can have a serious environmental impact. In recent years it has become clear that the environmental impact of acid mine drainage can be minimized by the construction of artificial wetlands that combine geochemistry and biological treatments. These systems are being designed for a range of wastewaters, most of which fall outside the scope of this article.

The precipitation of ferric hydroxide is typically biologically mediated by iron-oxidizing bacteria at acid pH, but is usually rather slow. Abiological oxidation becomes more important at pH values above 5, and this is usually much faster than the biological process. Most constructed wetlands for treating acid mine waters thus start with a zone designed to raise the pH. A bed of crushed limestone often suffices to raise the pH significantly, and it is important that this be kept anoxic to prevent rust precipitation on the limestone, which would prevent further production of alkalinity. Once the pH is near neutral, the water is discharged into an aerobic wetland to encourage the precipitation of iron and aluminum oxides, and the co-precipitation of arsenic, if this is present (100,101).

If heavy metals are present in the mine water, the iron-free water can be made to flow into an anaerobic part of the constructed wetland, where organic material, such as compost, manure or sawdust, provides reductants to sulfate-reducing bacteria that become established. These bacteria reduce sulfate to sulfide, which precipitates the heavy metal ions as insoluble sulfides. It is important that this part of the constructed wetland be kept anaerobic, to prevent oxidation and remobilization of the precipitated metals. For this reason, this part of the wetland is typically kept flooded and free of aquatic plants that might introduce oxygen through their roots (100). Finally, an aerobic algal mat can act as a polishing step to complete the removal of contaminants, particularly manganese (101,102). Mine drainage that is not acidic may not need such complicated systems and individual parts of the treatment train described above may suffice in some situations.

Soil. The first reported field trial of the use of hyperaccumulating plants to remove metals from a soil contaminated by sludge applications has been reported (103). The results were positive, but the rates of metal uptake suggest a time scale of decades for complete cleanup. Trials with higher biomass plants, such as *B. juncea*, are underway at several chromium and lead contaminated sites (88), but data are not yet available.

The bacterial reduction of Cr(VI) to Cr(III) discussed above is also being used to reduce the hazards of chromium in soils and water (104).

6. Conclusions

Bioremediation has been successfully used to treat a wide range of contaminants, including crude oils and refined petroleum products, halogenated solvents, pesticides, herbicides, military chemicals, and mine waters. Much of this success has come by small adjustments of the local environment to encourage the growth of remediating organisms. Fertilizer addition has been a successful treatment for terrestrial and marine oil spills, and the addition of co-substrates, particularly methane, has been a successful treatment for remediating halogenated solvents, such as trichloroethylene. Composting is proving to be a successful treatment for a range of contaminants, and constructed wetlands are successfully treating a range of wastewaters, including those emanating from mines.

One area where there is considerable disagreement between academic scientists and engineering practitioners of bioremediation is the area of bioaugmentation, the addition of selected microorganisms to a site to encourage biodegradation. Dozens of companies sell bacteria for this purpose and claim success in the field, but efforts to demonstrate effectiveness rigorously have met with little success (105,57). Perhaps the most startling test was performed by the U.S. EPA when testing potential inoculants for stimulating oil biodegradation in Alaska following the *Exxon Valdez* oil spill (106). Eight products were tested in small laboratory reactors that allowed substantial degradation of a test oil by the indigenous organisms of Prince William Sound. All eight microbial inocula had a greater stimulatory effect on alkane degradation, at least for the first 11 days, when they were sterilized by autoclaving prior to addition! This suggests that the indigenous organisms readily out-competed the added products, but that autoclaving the products released some trace nutrient that was able to stimulate the growth of the endogenous organisms.

Of course, bioaugmentation may prove more effective with contaminants that have only recently entered the biosphere, such as methyl-*tert*-butyl ether, or with organisms that have a dramatic selective advantage over indigenous organisms. For example, modern molecular biology may offer opportunities for moving effective degradation pathways into organisms native to a contaminated site, improving biodegradation pathways by broadening the substrate range of degradative enzymes, or removing regulatory constraints to maximize degradative activities. There are, however, many technical and regulatory hurdles to be surmounted before this potential can be tested.

Another area where there is controversy is in the role of surfactants. Many bacteria, particularly those that degrade hydrocarbons, produce surfactants as they grow. Some release them into the medium, others incorporate them into their cell exterior, and there have been elegant experiments to show that inhibiting the production of these compounds inhibits the ability of the bacteria to degrade oil. There have thus been many suggestions to add surfactants, either bacterial or synthetic, to stimulate biodegradation. This seems to be beneficial in the case of some oil dispersants at sea (12), but there have not yet been any clear demonstrations of efficacy on a large scale in soils. The role of surfactants in bioremediation is an area that requires further study.

Bioremediation has many advantages over other technologies, both in cost and in effectively destroying or extracting the pollutant. An important issue is thus when to consider it, and a series of questions may lead to the appropriate answer (see Table 4).

If the answers to the questions in Table 4 lead to the selection of bioremediation, it then becomes important to assess the success of the bioremediation strategy in achieving the clean-up criteria. A major disadvantage of bioremediation is that it is typically rather slower than competing technologies such as thermal treatments. How can regulators and responsible parties gain confidence during this time that success will indeed be achieved? The National Research Council (107) has recently addressed this issue, and suggested a three-fold strategy for “proving” bioremediation: (*1*) a documented loss of contaminants from the site; (*2*) laboratory tests showing the potential of endogenous microbes to

Table 4. **Will Bioremediation be a Suitable Treatment for a Site Contaminated with Organic, Nitrogenous, or Organic Contaminants?**

Organic

Is the contaminant biodegradable? If the contaminant is a complex mixture of components, are the individual chemical species biodegradable? If the contaminant has been at the site for some time, biodegradation of the most readily degradable components may have already occurred. Is the residual contamination biodegradable?
Are degrading organisms present at the site?
What is limiting their growth and activity? Can this be added effectively?
Are the levels of contaminant amenable to bioremediation? Are they toxic to microorganisms? Are they so abundant that even substantial microbial activity will take too long to clean the site?
Are the clean-up standards reasonable? Are biological processes known to degrade substrates down to the levels required?

Nitrogenous

Are appropriate microorganisms present at the site?
What is limiting their growth and activity? Can this be added effectively?
Are the levels of contaminant amenable to bioremediation? Are they toxic to microorganisms? Are they so abundant that even substantial microbial activity will take too long to clean the site?
Can the nitrogenous compound be used by plants?
Are the clean-up standards reasonable? Are biological processes known to degrade substrates down to the levels required?

Inorganic

Can the contaminant be made less hazardous by changing its redox state?
Can the contaminant be brought to a reactor or constructed wetland where biological systems, microbial or plant, can extract and immobilize the contaminant?
Can plants extract the contaminant from the soil matrix?
Are the clean-up standards reasonable? Are biological processes known to accumulate contaminants down to the levels required?

catalyze the reactions of interest; and (*3*) some evidence that this potential is achieved in the field.

Although laboratory tests to demonstrate the potential of endogenous organisms are relatively straightforward, the other two are often not as simple as they seem, especially in soils and sediments. Documenting the loss of contaminant is often difficult because of the heterogeneous distribution of the contaminant in the field; large numbers of samples may be needed in order to be able to detect statistically significant decreases in absolute concentration. Providing evidence that biodegradation has indeed been stimulated is also a challenge, but if the contaminant is a complex mixture, such as a crude oil, refined petroleum, or a mixture of polychlorinated biphenyls, the least degradable compounds in the mixture can be used as conserved internal markers for quantifying the degradation of the more degradable components.

For example, hopane (Fig. 12) is a conserved marker in crude oils in at least the early stages of biodegradation (up to 80% degradation) (108); its concentration increases in the residual oil as biodegradation proceeds. Basing estimates of biodegradation on hopane allowed us to quantify the effect of the successful bioremediation strategy following the *Exxon Valdez* oil-spill (14,15). Even in refined products, the least degradable detectable analyte can serve this role. Thus, the

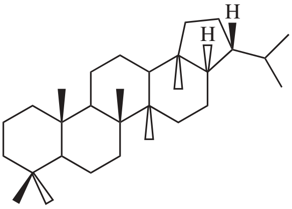


Fig. 12. 17α(*H*),21β(*H*)-hopane.

trimethyl-phenanthrenes can be used to estimate qualitatively the degradation of diesel oils in the environment, even though these molecules are themselves biodegradable (109). Of course, since these molecules are themselves biodegradable, estimates of the rates of biodegradation of more readily biodegradable compounds are systematically underestimated using this approach, but it can still provide very valuable information. The more halogenated polychlorinated biphenyls can serve a similar role in assessing the environmental fate of these products (55), and benzene is typically the last compound in BTEX plumes to be degraded, particularly under anaerobic conditions.

In situations where conserved internal markers cannot be used, such as in spills of essentially pure compounds, the evidence for enhanced biodegradation may have to be more indirect. Oxygen consumption, increases in microbial activity or population, and carbon dioxide evolution have all been used with success.

Finally, a caveat. Despite its documented success in many situations, bioremediation may not always be able to meet current clean-up criteria for a particular site. Some standards are so tight that they are essentially “detection limit” standards, and it is not clear that biological processes will be able to remove contaminants to such low levels. For example, the level of contaminant may be so low that it does not induce the microorganisms to produce the enzymes necessary for biodegradation. Or perhaps the contaminant is bound to soil or sediment particles in such a way that it is not available for biodegradation, although it is still extractable with aggressive solvents in analytical procedures. These are areas that require further research, but bioremediation will be more likely to fulfill its promise as an important tool in contaminated site remediation if there is progress towards standards based on bioavailability and net environmental benefit from the clean up, rather than on arbitrary absolute standards.

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