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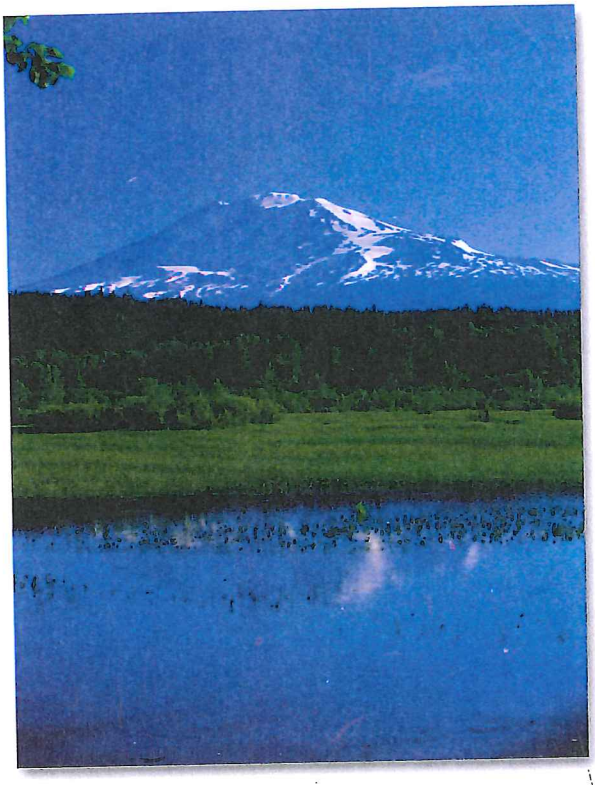

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ENVIRONMENTAL BIOTECHNOLOGY: PRINCIPLES AND APPLICATIONS

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BIOREMEDIATION

One of the newest and fastest growing applications of environmental biotechnology is *bioremediation.* Unlike most of the applications described in earlier chapters, bioremediation most often addresses treatment of contaminated solids, such as groundwater aquifers, soils, and sediments. This focus on decontaminating solids occurs because flowing waters poorly mobilize most of the common contaminants that reach these environments. Because the contaminants adsorb to the amply present solid surfaces or form a separate liquid phase, they are "trapped" in the solid matrix. Cleanup strategies that rely on flushing the contaminants out with water and treating the water (i.e., so-called pump-and-treat strategies) often are unsuccessful, even after years or decades of remediation by flushing (Travis and Doty, 1990; National Research Council, 1994).

Bioremediation overcomes the main deficiency of conventional cleanup approaches that rely on water flushing: Dissolution or desorption of the contaminants into water is too slow, since the water's contaminant-carrying capacity is very limited. With bioremediation, high densities of active microorganisms locate themselves close to the nonaqueous source of contamination. Within a short distance from its point of dissolution into the aqueous phase, the contaminant molecule is biodegraded. If the biodegradation is fast and occurs close enough to the nonaqueous source, biodegradation replenishes the water's ability to accept more dissolving or desorbing contaminant, bringing about accelerated dissolution/desorption, which decontaminates the solid phase.

Although bacteria are present in all soils, sediments, and aquifers, their naturally occurring numbers may be too small to bring about the rapid reaction needed to have enhanced dissolution/desorption. In that case, the strategy is to add to the contaminated environment the materials needed to allow growth of the bacteria active in degrading the target contaminant. These materials provide the substrates and nutrients required for the growth and maintenance of a high-density microbial population. The most commonly added materials normally are selected from among an electron-acceptor substrate (like oxygen), an electron-donor substrate (like a sugar or natural gas), inorganic nutrients (like nitrogen and phosphorus), and materials to help dissolve/desorb immobile substrates (like surfactants).

Bioremediation can be divided into three main classes: engineered in situ, intrinsic in situ, and engineered ex situ. The distinction between in situ and ex situ indicates whether the contaminated solids remain in place (i.e., in situ) during the bioremediation or are excavated and transferred to an aboveground treatment system (i.e., ex situ). Engineered bioremediation refers to employing engineering tools to greatly increase the input rates for the stimulating materials. In contrast, intrinsic bioremediation relies on the

intrinsically occurring rates of supply of substrates and nutrients, as well as the intrinsic population density of active microorganisms. The most important factor for any type of bioremediation is ensuring that the rate of biotransformation is fast enough to meet the cleanup objectives.

Because engineered bioremediation involves using engineered measures to add the materials necessary to *increase the biotransformation rates significantly,* the fast-enough criterion refers to increasing the biotransformation rates well above those that would occur without the engineered additions. In this way, the time required to clean up the contamination is substantially reduced. Engineered bioremediation is a cost-effective approach when one must meet a regulated deadline, minimize exposure risk, or facilitate a property sale.

Intrinsic bioremediation, on the other hand, relies on the intrinsic (or naturally developing) biological activity to *prevent the migration of contamination away from its source.* While intrinsic bioremediation does not accelerate the rate of cleanup, it prevents further spread of the contaminants. The natural supply of necessary materials, when coupled with the presence of appropriate microorganisms, must be fast enough to allow biodegradation of the contaminants before they can be transported a significant distance away from the source. A monitoring plan is essential to demonstrate that these intrinsic factors remain effective for preventing contaminant migration. It cannot be emphasized too much that intrinsic bioremediation is not a "do nothing" approach. Intrinsic bioremediation is sometimes called *natural attenuation,* a term that actually includes many naturally occurring mechanisms that can lead to a decrease in the concentration of a contaminant (National Research Council, 2000).

Prior to the design of a system for in situ bioremediation, several steps are required to ensure that the program is successful (Lee et al., 1988; National Research Council, 1993, 2000; Rittmann et al., 1994). These steps are needed to characterize the site in terms of hydrology, extent and type of contamination, intrinsic microbial activity, and intrinsic supply rates of key materials.

Although bioremediation is a new and uniquely challenging application of environmental biotechnology, it is founded on the same principles as are other applications. Because of the high specific surface area of aquifers, soils, and sediments, the microbial ecology is dominated by attached growth. Therefore, the principles and applications in Chapters 4 and 8 are particularly relevant here. Furthermore, the most common contaminants are the synthetic organic chemicals, whose biological detoxification is described in Chapter 14. The information in these closely related chapters is not repeated here, although short summaries are provided in order to provide the appropriate context for reviewing the other chapters.

This chapter focuses on the unique features of bioremediation. They are:

- The scope and characteristics of contamination
- Contaminant availability for biodegradation
- Treatability studies
- Engineering strategies for bioremediation
- Evaluating bioremediation

15.1 SCOPE AND CHARACTERISTICS OF CONTAMINANTS

The Office of Technology Assessment documented that over 200 substances have been detected in groundwater in the United States (OTA, 1984; Rittmann et al., 1994). These include organic chemicals, inorganic chemicals, biological organisms, and radionuclides. A summary is presented in Table 15.1.

Table 15.1 Summary of information presented on substances detected in groundwater

	Category of Compounds	Number of Entries	Examples[1]	Prevalent Uses[2]
1.	Aromatic Hydrocarbons	33	Benzene Ethylbenzene Toluene	• Dyestuffs • Solvents
2.	Oxygenated Hydrocarbons	24	Acetone 1,4 - Dioxane Phenols	• Solvents • Paints, varnishes
3.	Hydrocarbons with Specific Elements (e.g., with N, P, S, Cl, Br, I, F)	103	2,4-D Trichloroethene (TCE) Trichloroethanes (1,1,1 and 1,1,2 TCA) Trinitrotoluene (TNT)	• Pesticides • Solvents • Munitions
4.	Other Hydrocarbons	15	Kerosene Gasoline Lignin and Tannin	• Fuels
5.	Metals and Cations	27	Iron Chromium Arsenic	• Alloys • Electrical and electronics
6.	Nonmetals and Anions	10	Chloride Sulfate Ammonia	• Fertilizers • Foodadditives
7.	Microorganisms	2	Bacteria (e.g., coliforms) Viruses	—
8.	Radionuclides	19	Uranium 238 Tritium Radium 226	• Tracers • Medical applications

[1]The compounds taken for examples in each category are those with the highest concentrations.
[2]Uses listed are industrial applications that were commonly found under each category.
SOURCE: Rittmann et al., 1994.

Although the components listed in Table 15.1 are widely used by industry, agriculture, commerce, and households, poorly mobile contaminants trapped in the solid matrix may be underrepresented when only groundwater samples are taken. The fact that the groundwater is sampled tends to accentuate compounds that are more readily mobile. In addition, detection of substances in groundwater often is biased by sampling procedures, analytical detection limits, and the circumstances that prompted the detection and reporting (e.g., was it a planned activity, such as regulatory compliance, or in response to an apparent impact, such as a public complaint).

15.1.1 Organic Compounds

This chapter focuses on organic compounds, which are most often amenable to bioremediation and are the most commonly detected contaminants in groundwater. The Council on Environmental Quality (1981) compiled a list of the 33 synthetic organic contaminants most frequently found in drinking-water wells (see Table 15.2).

Table 15.2 The 33 synthetic organic contaminants reported to be most frequently found in drinking-water wells

Trichloroethene (TCE)	Isopropyl benzene
Toluene	1,1-Dichloroethene
1,1,1-Trichloroethane	1,2-Dichloroethane
Acetone	Bis (2-ethylhexyl) Phthalate
Methylene chloride	DBCP (Dibromochloropropane)
Dioxane	Trifluorotrichloroethane
Ethyl benzene	Dibromochloromethane
Tetrachloroethene	Vinyl chloride
Cyclohexane	Chloromethane
Chloroform	Butyl benzyl-phthalate
Di-n-butyl-phthalate	gamma-BHC (Lindane)
Carbon tetrachloride	1,1,2-Trichloroethane
Benzene	Bromoform
1,2-Dichloroethene	1,1-Dichloroethane
Ethylene dibromide (EDB)	alpha-BHC
Xylene	Parathion
	delta-BHC

SOURCE: CEQ, 1981; Rittmann et al., 1994.

Organic chemicals are associated with a variety of sources, including: subsurface percolation; injection wells; land application of wastewater, wastewater by-products, and hazardous waste; landfills; open dumps; residential disposal; surface impoundments; underground and aboveground storage tanks; pipelines; materials transport and transfer operations; pesticide and fertilizer applications; urban runoff; and oil and gas production wells (OTA, 1984).

Information on the chemical and physical properties of these organic compounds was given in Table 14.2. A more extensive list of parameters is given in Rittmann et al. (1994). Many of the compounds are hydrophobic, which is indicated by a relatively large octanol/water partition coefficient (i.e., log $K_{ow} > 1$) and limited water solubility (i.e., $< 10{,}000$ mg/l). Some of the compounds are highly volatile (i.e., Henry's constant $> 10^{-3}$ atm-m^3/mol), which allows them to partition to a gas phase when it is present.

Included in Table 14.2 are highly hydrophobic contaminants whose water solubility is low enough that they seldom are high-risk factors in terms of water contamination. Chief among them is the polycyclic aromatic hydrocarbons (PAHs) derived from petroleum and the polychlorinated biphenyls (PCBs). For example, the log K_{ow} values and water solubilities (in mg/l) for the simpler PAHs are 3.3 and 31 for two-ringed naphthalene, 4.6 and 1.1 for three-ringed phenanthrene; 4.5 and 0.075 for four-ringed anthracene, and 6.1 and 0.0038 for five-ringed benzo(a)pyrene. For the PCBs, some representative solubility values are 0.24 mg/l for Arochlor 1242 and 0.057 mg/l for Arochlor 1254; the last two digits indicate the percent Cl in the PCB mixture. This brief listing makes it clear that PAHs and PCBs are much less mobile than are most of the common groundwater contaminants listed in Table 14.2. While

their hydrophobicity greatly reduces their concentrations in the water, it also accentuates their longevity as trapped sources of contamination in the soil or sediment, as well as their ability to bioconcentrate in living organisms.

15.1.2 MIXTURES OF ORGANIC COMPOUNDS

In many situations, organic contaminants are found in mixtures. In many instances, the original contamination was a mixture of related components that co-exist normally in a commercial product. Key examples include the PCBs, various petroleum-distillation fractions used for a range of fuel and lubricating purposes, and PAHs found in tars, asphalts, and petroleum sludges. One of the most widely used PCB mixtures—Arochlor 1242—has 42 percent chlorine overall, but contains biphenyl congeners having 1 through 6 Cl substituents, with 80 percent having 3, 4, or 5 Cl substituents. The more lightly chlorinated congeners have higher water solubilities than do the more heavily chlorinated congeners, which means the "aged" PCB tends to gradually become enriched in more chlorinated components.

Table 15.3 lists the typical distillation fractions obtained from petroleum. Although the distillation fractions are much more homogenous than is crude petroleum, each fraction contains molecules having a substantial range of formula weights and the correlated large ranges of water solubilities and hydrophobicities.

Because of its widespread uses and improper disposal, gasoline is an excellent case to illustrate the complexity of these naturally occurring mixtures. Its chemical complexity depends on three variables: (1) the source of the crude oil from which the gasoline is refined, (2) how the gasoline is refined, and (3) the additives used. The total number of distinct hydrocarbons in a gasoline fraction might be of the order of 500; however, due to the predominance of certain hydrocarbons, a smaller number of components accounts for a relatively large portion of the gasoline fraction (Sachanen, 1954).

Table 15.3 Typical fractions obtained by distillation of petroleum

Boiling Range of Fraction	Number of Carbon Atoms per Molecule	Use
Below 20 °C	C_1–C_4	Natural gas, bottled gas, petrochemicals
20–60 °C	C_5–C_6	Petroleum ether, solvents
60–100 °C	C_6–C_7	Ligroin, solvents
40–200 °C	C_5–C_{10}	Gasoline (straight-run gasoline)
175–325 °C	C_{12}–C_{18}	Kerosene and jet fuel
250–400 °C	C_{12} and higher	Gas oil, fuel oil, and diesel oil
Nonvolatile liquids	C_{20} and higher	Refined mineral oil, lubricating oil, grease
Nonvolatile solids	C_{20} and higher	Paraffin wax, asphalt, and tar

SOURCE: Solomons, 1984; Rittmann et al., 1994.

Table 15.4 presents the composition of several typical straight-run gasolines (i.e., the gasoline fraction obtained by direct distillation of the crude petroleum) by class of hydrocarbon. Either alkanes or cycloalkanes dominate all of these straight-run gasolines, with those dominated by alkanes being more abundant; the aromatic hydrocarbons average below 20 percent by volume. In general, alkenes or unsaturated hydrocarbons are absent in straight-run gasolines. Despite these general trends, Table 15.4 shows that the source of the crude oil affects the chemical composition of the gasoline mixture.

The type of refining also plays a very important role in defining the gasoline mixture. Figure 15.1 shows that, in comparison with straight-run gasoline, thermal-cracked gasoline contains alkenes, increased aromatics, and decreased alkanes and cycloalkanes; catalytically cracked gasoline has alkenes, increased aromatics, and greatly decreased n-alkanes and cycloalkanes (Hill and Moxey, 1960). Further, unleaded gasolines generally have a higher aromatic hydrocarbon fraction than do leaded gasolines (Cline, Delfino, and Rao, 1991).

The most troublesome of the gasoline hydrocarbons are the single-ring aromatics: benzene, toluene, ethylbenzene, and the xylenes (BTEX). Unleaded gasoline typically contains 2–5 percent (by volume) of benzene, 6–7 percent of toluene, 5 percent of ethylbenzene, and 6–7 percent of xylenes. Compared to almost all other gasoline hydrocarbons, BTEX have by far the highest water solubility. Although the total for

Table 15.4 Chemical composition of straight-run gasolines

Origin & Boiling Range, °C	% by Volume Alkanes	Cycloalkanes	Aromatics
Pennsylvania, 40–200	70	22	8
Oklahoma City, 40–180	62	29	9
Ponca (Oklahoma), 55–180	50	40	10
East Texas, 45–200	50	41	9
West Texas, 80–180	47	33	20
Conroe (Texas), 50–200	35	39	26
Hastings (Texas), 50–200	27	67	6
Michigan, 45–200	74	18	8
Rodessa (Louisiana), 45–160	72	20	8
Santa Fe Springs (Calif.), 45–150	41	50	9
Kettleman (Calif.), 45–150	48	45	7
Huntington Beach (Calif.), 50–210	35	54	11
Turner Valley (Canada), 45–200	51	35	14
Altamira (Mexico), 40–200	49	36	14
Portero (Mexico), 50–200	57	35	8
Bucsani (Romania), 50–150	56	32	12
Baku-Surachany, 60–200	27	64	9
Baku-Bibieibat, 60–200	29	63	8
Grozny, New Field, 45–200	64	29	7
Iran, 45–200	70	21	9
Kuwait, 40–200	72	20	8

SOURCE: Sachanen, 1954; Rittmann et al., 1994.

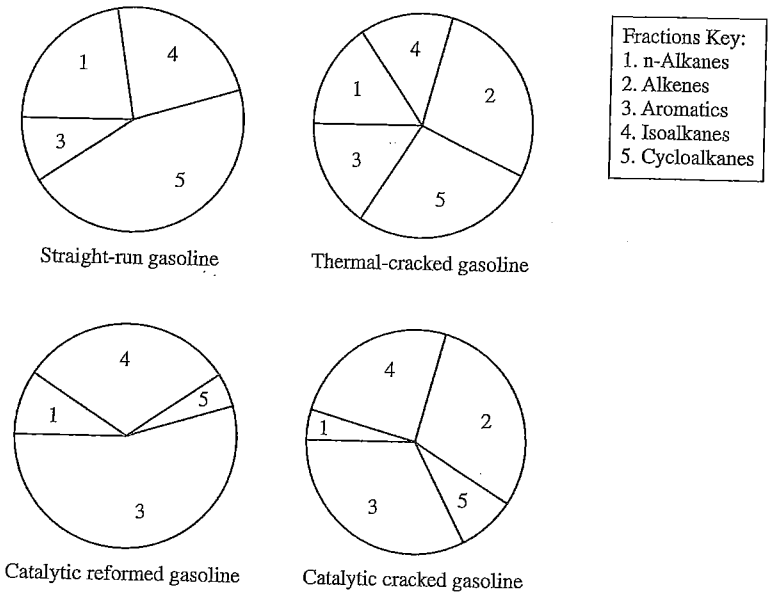

Figure 15.1 Hydrocarbon composition of gasoline components. SOURCE: Hill and Moxey, 1960.

BTEX is roughly 20 percent by volume, the water solubility of BTEX causes them to be the prime water pollutants.

The composition of gasoline is further complicated by additives, which are added for several purposes: (1) antiknock compounds, (2) antioxidants, (3) metal deactivators, (4) antirust agents, (5) antistall agents, (6) antipreignition agents, (7) upper-cylinder lubricants, (8) alcohols (e.g, ethanol), and (9) oxygenates (e.g., MTBE). Table 15.5 summarizes typical additives (Hill and Moxey, 1960; Lane, 1980; Cline et al., 1991; Rittmann et al., 1994). With the exception of alcohols used for gasohol,

Table 15.5 Additives typical in gasolines

Additive Type	Typical Chemicals Used	% by Volume
Antiknock agents	Tetraethyl lead, tetramethyl lead	0.08
	tert-butanol, methyl *tert*-butyl ether	< 3.5, < 15%
Antioxidants	Aminophenols, orthoalkylated phenols	< 0.006
Metal deactivators	N, N′-disalicylidene- 1,2-diaminopropane	0.0004
Antirust agents	Fatty acid amines, amine phosphates	0.0005
Antistall agents	Alcohols, glycols	1–2
Antipreignition agents	Alkyl phosphates	0.02
Upper-cylinder lubricants	Light mineral oils, Cyclo-paraffinic distillates	0.2–0.5
Alcohols	Ethanol	< 40
Oxygenates	MTBE (methyl-*tert*-butyl ether)	< 5

antistall, or antiknock purposes and methyl-*tert*-butyl ether (MTBE) used for antiknock and oxygenate purposes, the additives are present in very low percentages and seldom affect groundwater. On the other hand, the alcohols and MTBE can be major groundwater contaminants, along with BTEX. In fact, the massive introduction of MTBE in gasoline as an oxygenate to reduce smog in California has greatly worsened the problems of groundwater contamination in that state. MTBE is being removed from gasoline in the United States in order to prevent more groundwater contamination.

15.1.3 MIXTURES CREATED BY CODISPOSAL

In many instances, subsurface contamination is a mixture of disparate materials whose main (or only) connection is that they were codisposed of at a single site. These codisposal mixtures can include a wide range of organic compounds that do not necessarily occur together naturally, as well as inorganic chemicals.

A common situation of codisposal is the mixture of organic and inorganic materials in sanitary landfills and in their leachates. Table 15.6 presents a list of various inorganic constituents in leachate from sanitary landfills and representative ranges of concentrations. Large numbers of inorganic contaminants can be found in leachate,

Table 15.6 Representative ranges for various inorganic constituents in leachate from sanitary landfills

Parameter	Representative Range (mg/L)
K^+	200–1,000
Na^+	200–1,200
Ca^{2+}	100–3,000
Mg^{2+}	100–1,500
Cl^-	300–3,000
SO_4^{2-}	10–1,000
Alkalinity	500–10,000
Fe (total)	1–1,000
Mn	0.01–100
Cu	< 10
Ni	0.01–1
Zn	01–100
Pb	< 5
Hg	< 0.2
NO_3^-	0.1–10
NH_4^+	10–1,000
P as PO_4	1–100
Organic nitrogen	10–1,000
Total dissolved organic carbon	10–1,000
COD (chemical oxygen demand)	1,000–90,000
Total dissolved solids	5,000–40,000
pH	4–8

SOURCE: Freeze and Cherry, 1979; Rittmann et al., 1994.
The ranges for the constituents came from many leachate sources and are not necessarily consistent with each other.

some at high concentrations; many organic contaminants also can be found in leachate and cause the relatively high total dissolved organic carbon and COD values. The high concentration of inorganic ions can affect electron-acceptor availability (e.g., SO_4^{2-} and NO_3^-), precipitation potential (e.g., PO_4^{3-}, CO_3^{2-} in alkalinity), and toxicity (e.g., heavy metals, sulfides). A large fraction of the COD often is volatile acids, which tend to lower the pH, but also are good primary substrates. Biodegradation of the volatile acids removes their acidity and allows the pH to increase.

Robertson, Toussaint, and Jorque (1974) investigated the organic compounds leached into the groundwater from a landfill near Norman, Oklahoma. Refuse had been deposited below or near the water table of a sandy aquifer. They found low levels of more than 40 organic compounds in the groundwater and concluded that the source of many of these compounds was the leaching of manufactured products discarded within the landfill. In addition to constituents derived from solid wastes, many landfill leachates also contain toxic constituents emanating from liquid industrial wastes located within the landfill.

Many industrial, commercial, and military operations led to exotic mixtures of organic and inorganic contaminants. One example of groundwater pollution resulting from industrial/military operations is for the McClellan Air Force Base, near Sacramento, California. Table 15.7 shows a wide range of solvents, fuel components, and metals that resulted from wastes containing solvents, paint, fuel oil, and electroplating residues that were disposed of by landfilling and burning in pits.

Table 15.7 Volatile and nonvolatile organic compounds and trace metals found in groundwater at McClellan Air Force Base, Sacramento, CA

Compound	Concentration (mg/L)
1,1-Dichloroethene	60
Acetone	35
Methyl ethyl ketone	25
1,1,1-Trichloroethane	12
Trichloroethene	11
Methylene chloride	5
Methyl isobutyl ketone	3.7
Vinyl chloride	2.5
Dichlorobenzene	0.17
Benzene	0.68
1,1-Dichloroethane	0.25
Toluene	0.08
Phenols	0.5
Tetrachloroethane	0.07
Trans-1,2-dichloroethene	0.2
Chromium	0.12
Nickel	0.10
Zinc	0.073
Lead	0.093
Selenium	0.049
Cadmium	0.012

SOURCE: Pitra and McKenzie, 1990; Rittmann et al., 1994.

Major chemical-manufacturing facilities often have disposal areas contaminated over many years with waste solvents, sludges, unacceptable products, and other residues. The whole range of common contaminants often is present. In addition, very low solubility sludges and "bottoms" create a poorly characterized "goop" or "gumbo."

The Department of Energy (DOE) has responsibility for managing the sites where nuclear weapons were produced. DOE sites are unique in that the contamination of the subsurface often involves complex mixtures of organic and inorganic chemicals, including short- and long-lived radionuclides (USDOE, 1990). Important individual contaminants found at DOE sites are listed in Table 15.8. These inorganic and organic species have been found in a variety of combinations. An extremely important interaction is complexation between the heavy-metal radionuclides and the strong chelating agents, such as EDTA and NTA. The degree of complexation with

Table 15.8 DOE site contaminants

Inorganic Species	Organic Species
Radionuclides	Organic Contaminants
Plutonium-238,239	Chlorinated hydrocarbons
Americium-241	Methyl ethyl ketone
Thorium	Cyclohexanone
Uranium-232,234, 238	Tetraphenyl boron
Technetium-99	Polychlorinated biphenyls (PCBs)
Strontium-90	Select polycyclic aromatic
Cesium-134, 137	hydrocarbons (PAHs)
Cobalt-60	Tributyl phosphate
Europium-152, 154, 155	Toluene
Nickel-63	Benzene
Iodine-129	Kerosene
Neptunium-237	
Radium	
Metals	Facilitators[a]
Lead	Aliphatic Organic acids
Nickel	(citric, lactic, succinic, oxalic to octadecanoic)
Chromium	Chelating agents (EDTA, NTA, DTPA, HEDTA,
Copper	TTA, Di-2-ethyl HPA)
Mercury	Aromatic acids (humic acids and subunits)
Silver	Solvent, diluent, and chelate radiolysis
Bismuth	fragments
Palladium	
Aluminum	
Others	
Carbon-nitrogen compounds	
Nitrite	
Nitrate	

[a]Facilitators are organic compounds that interact with and modify metal/radionuclide geochemical behavior.
SOURCE: USDOE, 1990; Riffmann et al., 1994.

the chelators controls the mobility of the radionuclides, while the biodegradation of the chelators is affected by their complexation to the heavy metals.

15.2 BIODEGRADABILITY

Fortunately, essentially all of the common organic contaminants found in groundwater are biodegradable, provided the proper conditions exist. Chapter 14 reviewed the biochemical and physiological factors controlling the biodegradation of these kinds of hazardous organic pollutants.

A brief summation of items most relevant to bioremediation is:

- The aliphatic and aromatic hydrocarbons are readily biodegradable by a range of aerobic bacteria and fungi. The key is that molecular O_2 is needed to activate the molecules via initial oxygenation reactions.
- Evidence of anaerobic biodegradation of aromatic hydrocarbons is growing. Anaerobic biodegradation rates are slower than aerobic rates, but they can be important when fast kinetics are not essential.
- Most halogenated aliphatics can be reductively dehalogenated, although the rate appears to slow as the halogen substituents are removed.
- Highly chlorinated aromatics, including PCBs, can be reductively dehalogenated to less halogenated species.
- Lightly halogenated aromatics can be aerobically biodegraded via initial oxygenation reactions.
- Many of the common organic contaminants show inhibitory effects on microorganism growth and metabolism. Due to their strongly hydrophobic nature, many of the inhibitory responses are caused by interactions with the cell membrane. In some cases, intermediate products of metabolism can be more toxic than the original contaminant.

15.3 CONTAMINANT AVAILABILITY FOR BIODEGRADATION

A factor that frequently limits the rate of bioremediation is *substrate unavailability,* or when only a small fraction of all the substrate molecules is truly water soluble and, therefore, available to the microorganisms. Two phenomena that limit substrate availability are strong sorption to surfaces and formation of a nonaqueous phase. Both factors keep aqueous-phase concentrations low. Low concentration slows degradation kinetics in general and may also prevent biomass accumulation by being lower than S_{min} or by preventing enzyme derepression or induction. However, low aqueous-phase concentrations sometimes are beneficial for biodegradation, particularly when the compound is inhibitory.

15.3.1 SORPTION TO SURFACES

The effect of sorption on biodegradation in the subsurface can be complex. In some cases, sorption of substrates stimulates biodegradation, but in many cases it retards biodegradation. In order to come to some understanding of the interactions between biodegradation and sorption, it is helpful to review sorption and how solid surfaces affect microbial physiology.

What Controls Sorption For most of the organic molecules of importance to bioremediation, their sorption to solid surfaces is controlled primarily by their hydrophobic nature. Being nonpolar and of low solubility, they are thermodynamically driven out of aqueous solution by entropy effects. In other words, having these nonpolar solutes dissolved in water requires a restructuring of the water molecules to a higher free-energy state. Ejecting the nonpolar solutes from water solution reduces the free energy of the system. This effect is magnified when the surface to which the nonpolar molecule adsorbs is itself hydrophobic.

A useful means for quantifying this adsorption effect for nonpolar, organic solutes is the linear partitioning model:

$$\psi = K_p C \qquad \textbf{[15.1]}$$

in which C is the aqueous-phase concentration of the solute [ML^{-3}], ψ is the adsorption density of the sorbed solute [M/M], and K_p is the linear partition coefficient [L^3/M]. The linear-partition model assumes equilibrium between C and ψ. The K_p value often is related directly to the hydrophobicity of the solute and the surface onto which it adsorbs. Based on the work of Karickhoff, Brown, and Scott (1979), K_p can be expressed as

$$K_p = 0.63\ K_{ow} f_{oc} \varepsilon / \rho_b \qquad \textbf{[15.2]}$$

in which K_{ow} is the solute octanol/water partition coefficient, which has units of volume water/volume octanol; f_{oc} is the fraction by mass of organic carbon in the solid phase; 0.63 is an empirical conversion factor that has units mass solid-volume octanol/(mass-volume water); ρ_b is the bulk density, or mass solids/total volume; and ε is the porosity, or water volume/total volume.

McCarty et al. (1985) derived a relationship for the retardation factor, R, which is the ratio of the advective water velocity to the velocity of the contaminant:

$$R = 1 + (\rho_b/\varepsilon)K_p = 1 + 0.63\ K_{ow} f_{oc} \qquad \textbf{[15.3]}$$

A large R value means that the solute is strongly immobilized by adsorption. For example, anthracene ($K_{ow} = 10^{4.54}$) would have an R of 1,100 in a highly organic soil or sediment ($f_{oc} = 0.05$). On the other hand, TCE ($K_{ow} = 10^{2.29}$) would have an R of only 1.12 in a sandy aquifer with a low organic content ($f_{oc} = 0.001$). Likewise, the adsorption densities (ψ) would be quite different: A 10-μg/l aqueous-phase concentration would give ψ values of 1,370 and 0.15 μg/g soil for anthracene and TCE, respectively ($\rho_b = 2$ kg/l and $\varepsilon = 0.25$).

Influences of Solid Surfaces on Microbial Physiology Studies of the naturally occurring microbes in aquifers show that the numbers of microorganisms attached to aquifer solids usually are 10 to 1,000 times greater than those free-living in the groundwater. The ratio may be higher in soils and sediments. Although bacterial attachment to surfaces undoubtedly can affect the activity of the bacteria, what the effect will be cannot be predicted readily. Van Loosdrecht, Lyklema, Norde, and Zehnder (1990) summarized the apparently contradictory influences of solid surfaces on microbial behavior: increased growth rate, decreased growth rate, increased assimilation and decreased respiration rates, decreased assimilation, increased respiration, increased adhesion of active cells, higher activity of attached cells, decreased substrate utilization, lower substrate affinity, change in pH optimum, difference in fermentation pattern, increased productivity, decreased mortality, and no effect.

Some confusion can be attributed to a failure to distinguish between direct and indirect influences of solid surfaces. A direct influence results from the presence of a surface itself. An indirect influence occurs because the aggregation of microorganisms alters their environment, such as concentrations of substrate or pH. One reason for confusion comes about because bacteria attaching to living surfaces, such as with plants and animals, often attach at and respond to specific receptors. Whereas these highly specific attachments trigger profound alterations to the physiology of the attaching microbe, as well as its host, the nonliving surfaces of soil and sediments cannot evoke the same kind of reaction. Thus, any effects on microbial physiology in environmental settings are normally the result of indirect effects via changes to the cells' local environment. These changes in local environment can include concentration gradients for substrate and pH. They also can include signaling molecules that transfer between different microorganisms.

Biodegradation of Sorbed Molecules Sorption can cause either decreased or increased biodegradation, and the observed effects depend on the characteristics of the compound, the solid phase, and the microorganisms. One obvious way in which sorption can slow biodegradation rates is simply by decreasing the aqueous-phase concentration. Since most bacteria utilize only dissolved solutes, adsorption becomes a competing sink. In such cases, compounds having large R values see a dramatic decrease in the biodegradation rate per unit of contaminant present, since most of the solute is adsorbed to the surface and inaccessible for direct biodegradation. If equilibrium partitioning holds, only $1/R$ of the total compound present is available for biodegradation. For example, if anthracene had a first-order degradation rate constant of 0.1/d for solute strictly in the dissolved state, its first-order rate for all anthracene would drop to only 0.000091/d if R were 1,100, as in the example above. Clearly, strong sorption can greatly slow degradation of highly hydrophobic chemicals.

When sorption is important, the rate of biodegradation can, in some cases, be controlled by the rate of desorption. As a first approximation, the desorption rate can be described by a single-resistance, mass-transport model,

$$J_d = k_{mt}[(\psi/K_p) - C] \qquad \textbf{[15.4]}$$

in which J_d is the desorption flux [$ML^{-2}\ T^{-1}$] and k_{mt} is a mass-transport coefficient [LT^{-1}]. In a general sense, biodegradation acts to increase J_d by keeping C low. When biodegradation (or another sink mechanism, such as advection or dispersion) forces C to approach zero, the rate of biodegradation then becomes mass-transport limited, and C and ψ are not in equilibrium (Seagren, Rittmann, and Valocchi, 1993, 1994).

When desorption is mass-transport limited, k_{mt} becomes the critical factor controlling the rate of bioremediation. Unfortunately, k_{mt} is a very poorly defined parameter for real aquifer, soil, or sediment systems. In many circumstances, k_{mt} is not controlled by a single mass-transfer step. Transport steps that may be acting include: movement from inside a porous solid (such as between the layers of clays or the interior of organic aggregates) to the water/solid interface, transfer across the interface, transfer across a nonturbulent boundary layer surrounding the solid particle, and transfer in the bulk liquid. This situation is complicated when the microorganisms are located at the solid/water interface and in the boundary layer; having biodegradation reactions in these locations violates the assumption of linear gradients that is inherent to Equation 15.4. This complication can be overcome by defining C to be adjacent to the interface. However, the interfacial C cannot be measured, and the complexity of the modeling increases greatly (Seagren et al., 1993, 1994, 2000).

Irreversible sorption is an extreme case of mass-transport limitation. Adsorption of solutes inside the crystalline lattice of clay minerals can lead to irreversible adsorption if the lattice structure collapses after adsorption takes place. Blocking of intra-aggregate pores by strongly adsorbed molecules also can lead to irreversible sorption. Polymerization of adsorbed organic molecules, particularly phenolics, also has been implicated as a means for making adsorption essentially irreversible. In this case, K_p effectively increases, and pore blockage also may result.

Bioremediation can be enhanced by adsorption when the compound is toxic. Sequestering the inhibitor and creating a nontoxic aqueous-phase concentration can lead to microbial growth and substrate metabolism. This beneficial sequestering is most effective when the solid has a strong intraparticle adsorption capacity. Sorption also can be beneficial when the sorbed substrate is stored and later released for biodegradation. This "warehousing" approach allows the microorganisms to accumulate to a high density and then modulate the desorption by creating a low value of C (van Loosdrecht et al., 1990; Chang and Rittmann 1987; Rittmann, Schwarz, and Sáez, 2000).

Although sorption can have various positive and negative influences on biodegradation, its relevance is accentuated for soils with high organic carbon or clay content and aggregates having internal porosity. However, in situ bioremediation generally requires fluid-permeable deposits that encompass materials ranging from silty sands to gravel. These materials may have little organic carbon content, cation exchange capacity, or internal pores.

15.3.2 FORMATION OF A NONAQUEOUS PHASE

A second way in which compounds show limited availability as substrates is formation into *nonaqueous phase liquids (NAPLs)*—fluids that are essentially immiscible with water and migrate through the subsurface as a separate phase. This category

of fluids includes petroleum, its refined fluid derivatives, and aliphatic chlorinated hydrocarbons. These fluids become trapped as residual saturation and lenses in the unsaturated and saturated zone. In the case of NAPLs less dense than water (LNAPLs, e.g., petroleum products), the pools can form at the top of the water table. Pools or residuals migrate to the aquifer bottom in the case of NAPLs more dense than water (DNAPLs, e.g., aliphatic chlorinated hydrocarbons). This trapped NAPL serves as a long-term source of groundwater contamination via dissolution. The focus here is on petroleum-product NAPLs, although most of the subjects reviewed apply equally to other NAPLs or NAPL mixtures.

Solubility Although NAPLs are defined as *immiscible* in water, they are, in general, slightly soluble in water. Table 15.9 lists selected gasoline hydrocarbons and their pure-compound water solubilities. The lowest solubilities (< 1 mg/l) are associated with the normal and branched alkanes. However, aromatics have solubilities greater than 100 mg/l.

Because a gasoline NAPL is a mixture, the actual equilibrium solubilities are substantially reduced from the pure-compound solubilities shown in Table 15.9. In most cases, the water solubility for mixtures of hydrocarbons follows ideal-solution theory, which says that the equilibrium solute concentration, C_i, of the ith component in an NAPL mixture is proportional to the product of its pure-compound solubility,

Table 15.9 Solubility of selected pure hydrocarbons in water at room temperature

Hydrocarbon	mg/L in Distilled Water
n-Alkanes	
n-propane	62
n-pentane	39
n-hexane	9.5
n-octane	0.7
Branched Alkanes	
2-methylbutane	48
2,3-dimethylhexane	0.13
Cycloalkanes	
cyclopentane	156
cyclohexane	55
methylcyclopentane	42
methylcyclohexane	14
Aromatics	
benzene	1780
ethylbenzene	152
toluene	515
o-xylene	175
p-xylene	198

SOURCE: Verschueren, 1983; Rittmann et al., 1994.

S_i, and its mole fraction in the mixture,

$$C_i = X_i S_i \qquad [15.5]$$

in which X_i is the component's mole fraction in the NAPL.

Aromatic components in gasoline, such as BTEX, have the highest S_i values, and they also are present at relatively large mole fractions. Therefore, they dissolve most rapidly into the water. Although this causes BTEX to be the most prevalent water pollutant, it also makes BTEX the most accessible for biodegradation. When combined with the fact that BTEX compounds are readily biodegradable under aerobic conditions, this relative propensity to become water-soluble has made bioremediation of BTEX by far the most widely practiced type of bioremediation.

Because the C_i values for the non-BTEX hydrocarbons in NAPLs are very low, sequential dissolution and biodegradation of the truly dissolved component is a slow means for the hydrocarbon-degrading microorganisms to make their substrate accessible. To maximize their rate of access to hydrocarbon substrates, some bacteria and fungi able to degrade long-chain aliphatics adhere to the hydrocarbon-NAPL interface and participate in a form of "direct uptake" of the substrates that they can metabolize. [See Rittmann et al. (1994) for a review of the adherence phenomenon.] Adherent bacteria are predominant when the amount of surface area is small, a common situation when mechanical agitation cannot be provided to emulsify the NAPL. The exact mechanism by which adhesion promotes substrate accessibility is not clear. By being very close to the NAPL/water interface, adhering microorganisms minimize the diffusion distance from the NAPL to the cell, thereby reducing the mass-transport resistance. Some believe that the hydrocarbon substrate is transferred directly into the cell without dissolving into the liquid.

When direct contact is important for increasing hydrocarbon biodegradation rates, the amount of NAPL/water surface area is critical. Emulsification or pseudo-solubilization is a way in which the surface area can be increased. Agitation, use of synthetic surfactants, production of microbial metabolites having surface-active properties (bio surfactants), or a combination can bring them about. Ex situ technologies are most effective for this surface-enhancing approach. The lack of agitation and difficulties contacting the NAPL with the surfactant make in situ application of surface enhancement problematical.

For in situ bioremediation, surfactants can be used to mobilize residual NAPL. The hydrocarbons in the NAPL are extracted into surfactant micelles (i.e., colloids of nonaqueous-phase surfactant) formed when the surfactant's critical micelle concentration (CMC) is exceeded. Mobilization of hydrocarbons by micelles may increase or decrease biodegradation. Most field experiences indicate that the application of micellular surfactants has a negative effect on biodegradation (Staps, 1990). Reduced biodegradation can be attributed to toxicity from the surfactant or to a further reduction in the dissolved concentration.

The use of surfactants to enhance biodegradation has been confused by the unfortunate use of the term "solubilize" to refer to the partitioning of hydrocarbons into colloidal micelles of surfactant. The hydrocarbon molecules are mobilized with the micelles, which often move with the water phase. However, these components are

not truly soluble, because they exist inside the surfactant phase. In fact, truly soluble components often have reduced concentrations due to their partitioning into the micelles. The term "solubilization" is false when biodegradation of soluble components is considered. The correct term is mobilization, and the components are mobilized in a colloidal, nonaqueous phase.

Mobilization in micelles increases biodegradation rates when the micellular hydrocarbon is more available to the microorganism than it would be if it remained in its original NAPL. Use of surfactants in ex situ bioremediation can greatly accelerate biodegradation when the nonaqueous-phase surface area is greatly increased. Most probably, the surface area is increased due to the combination of a decrease in surface tension and energy inputs, not from transfer of the hydrocarbons to surfactant micelles.

Surfactants, either biological or synthetic, could be used to mobilize hydrocarbons for the purpose of withdrawing them from the aquifer for treatment above ground (Lee et al., 1988). A danger concomitant with surfactant application to enhance contaminant removal in the subsurface is that the spreading of contaminants to previously uncontaminated parts of the aquifer could exacerbate the contamination problems.

15.4 TREATABILITY STUDIES

Before an in situ bioremediation project is attempted in the field, treatability studies may need to be performed (Lee et al., 1988; Thomas and Ward, 1989; Rittmann et al., 1994). The overall goal of treatability studies is to verify that the in situ approach has a high likelihood of succeeding. The number and type of treatability studies depend upon the characteristics of the site and the contaminants. In general, the level of effort that needs to be expended on treatability studies is proportional to the uncertainty about site and contaminant characteristics: When uncertainty is low, little effort may ensure success, but extensive efforts are needed when uncertainty is high.

Table 15.10 presents a hierarchical scheme for determining what kind of treatability study is needed. Each level is used to answer different kinds of questions. A treatability study should be carried out for each level for which answers are not known from other sources.

Level 1 is used to answer the fundamental question, "Is biodegradation a feasible option with the contaminants at the site?" Level 1 tests are not needed when the biodegradabilities of the contaminants are established from the literature or from previous bioremediation experience. Level 1 is needed most often when the contaminants are unusual, are not identified (e.g., they are only peaks on a chromatogram), or occur in mixtures that may be inhibitory (e.g., with toxic organic compounds, heavy metals, or high salts) or incompatible (e.g., some contaminants are known only to be degraded aerobically, while others are degraded only anaerobically).

The techniques for Level 1 studies ought to be as simple as possible. The tube microcosms of Wilson and Noonan (1984), serum bottles (Shelton and Tiedje, 1984), and hypovials (Bouwer, Rittmann, and McCarty, 1981) usually are satisfactory. The

Table 15.10 Hierarchical approach to determining what kind of treatability studies are needed to ensure success with in situ bioremediation

Level	Goals	Approach
1	Determine if biodegradation is an option	Simple laboratory microcosms, "yes" or "no" answer on biodegradability
2	Evaluate biodegradation nonidealities, such as concentration dependence of kinetics, inhibition, sorption, dissolution, mixed substrates, intermediates formation, and the need for other substrates or nutrients	Laboratory microcosms of all types, including hypovials, shake flasks, serum vials, columns, and slurries
3	Evaluate site-specific issues, such as hydrogeologic conditions and heterogeneity	Field pilot study

SOURCE: Rittmann et al., 1994.

keys are to avoid complications with volatilization and adsorption and to ensure that the reaction conditions in the microcosms are optimal for the reactions being evaluated. Usually, the disappearance of the target components, in comparison with abiotic controls, is sufficient to give the desired "yes" or "no" answer on the feasibility of biodegradation.

Once feasibility is established, Level 2 studies are needed to provide estimates of the rate of reaction and to identify complicating factors. Depending on the situation, Level 2 studies can address a wide range of questions. Some appropriate questions are:

- What is the functional relationship between the concentration of contaminant and its biodegradation rate?
- How do concentrations of other substrates, such as the primary electron donor and acceptor, affect the reaction rate?
- What is the stoichiometry needed for addition of substrates and nutrients? In particular, the stoichiometric needs should be established with knowledge of how the added material is going to be used; for example, as a growth-supporting substrate or nutrient, as a cosubstrate, or as an electron source or sink.
- Will environmental factors, such as low temperature, affect the rates?
- Do intermediates form, especially hazardous ones?
- Do the contaminants need to desorb or dissolve before they can be degraded? Can these rates be increased by addition of surface-active agents?
- Do toxicants, including intermediates and other substrates, interfere with biodegradation?

Level 2 experiments can be performed in many types of systems, including all those used for Level 1, columns, soil beds, and slurry reactors. The design of the system should depend on the specific question being asked and on sampling requirements. Even when the experimental system is the same as for Level 1, the intensity for

sampling and analysis must be much greater for Level 2. Whenever possible, Level 2 studies should be performed with actual soil, aquifer, and/or groundwater samples.

Level 2 studies should yield reasonable estimates of the rate of reaction in situ; limitations from inhibition, solubility, or intermediates; and needs for substrates and nutrients. This information, when combined with knowledge of the site's hydrogeology and contamination, should allow design of a field program based on sound science and engineering.

Level 3, field pilot testing, is used to answer the question, "Can the in situ bioremediation succeed under the site-specific conditions actually existing?" Real-world factors of heterogeneity and poor permeability can prevent success in the field, even though microbiological factors are positive. Level 3 testing may not need to be performed when site characterization shows that the site is hydrogeologically simple and highly permeable.

A Level-3 test normally is performed on a small portion of the contaminated site. The pilot location should be representative of the overall site in terms of hydrogeology and contamination. In situ conditions for the pilot test should simulate the proposed bioremediation scheme. The pilot site must be large enough that realistic complications are encountered. Simulation modeling of the transport and microbiological aspects of the bioremediation is recommended as part of the design and evaluation of the pilot study.

15.5 ENGINEERING STRATEGIES FOR BIOREMEDIATION

15.5.1 SITE CHARACTERIZATION

The first step in any bioremediation is site characterization in terms of its geology, hydrology, geochemistry, type and distribution of contamination, and microbiology. Site assessments performed to meet regulatory requirements typically do not provide nearly enough or the right kind of information for evaluating the potential for bioremediation. Therefore, site characterization needs to be tailored for bioremediation.

Critical information includes:

- The nature of the geological deposits, including the presence of fractures, thickness and extent of aquifers, location of confining units, and particle-size distribution
- The porosity (ε), hydraulic conductivity (K_{hyd}), and the variability of these parameters for the water-transmitting units
- The direction and slope of the naturally occurring hydraulic gradient
- As much information as is possible to obtain on the location, type, and mass of the contamination source; and the extent and concentration distribution of a contaminant plume

- Intrinsic supply and consumption rates of electron acceptors, nutrients, alkalinity, and other geochemical species affecting biodegradation
- Information on microbiological activity at least at Level 1 under Treatability Studies

15.5.2 ENGINEERED IN SITU BIOREMEDIATION

Because engineered in situ bioremediation is used to accelerate biologically driven removal of contaminants trapped in the solid phase, its success depends upon being able to achieve substantially increased inputs of stimulating materials. Thus, the ideal site has these features:

1. The hydraulic conductivity is relatively homogeneous, isotropic, and large (i.e., greater than about 10^{-5} m/s).
2. Residual concentrations are not excessive; NAPL concentrations greater than about 10 g/kg reduce aquifer permeability and microorganism access to the biodegradable contaminants.
3. The contamination is relatively shallow, in order to minimize costs of drilling and sampling.

The permeability, which controls the hydraulic conductivity, probably is the most important factor. Most successful projects have been in sandy environments having K_{hyd} values of 10^{-5} to 10^{-3} m/s (Staps, 1990). Heterogeneity is the most prevalent deviation from the ideal situation. Even when a formation has a suitably large K_{hyd}, that K_{hyd} often is an "effective," or spatially averaged value. Heterogeneity in K_{hyd} can have a significant impact on engineered bioremediation. Contaminants trapped in regions of relatively low K_{hyd} tend to be by-passed by the fluid flows bringing stimulatory materials.

Engineered in situ bioremediation systems can be classified according to the means by which stimulatory materials are added. Although technical details depend strongly on site-specific conditions and are advancing rapidly, these broad classifications are valuable for identifying what approaches have merit. The following methods are being applied successfully for in situ bioremediation (mainly) of petroleum hydrocarbons.

Bioventing When the contaminants are trapped in the unsaturated zone (also called the vadose zone) above the water table, the easiest way to supply O_2 is by pulling air through the unsaturated soil. This technique, called *bioventing,* is illustrated in Figure 15.2. Vacuum pumps create negative pressure that sweeps air through the soil and past the contaminated soil. The spacing of the vacuum points depends on the soil's permeability and the applied vacuum. Enough vacuum points are needed to ensure that O_2 is delivered to all contaminated units.

Passing air through the unsaturated zone evaporates moisture and can desiccate the soil enough that microbiological activity is slowed or even prevented. Therefore, Figure 15.2 illustrates how water is added to the *biologically active zone* (BAZ) by infiltrating water. Infiltration can be accomplished by spraying or flooding the surface

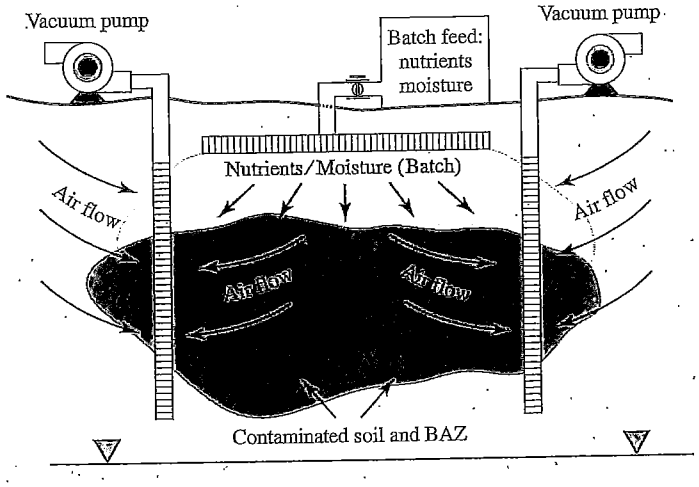

Figure 15.2 Bioventing is used to bioremediate contaminants trapped above the water table, which is indicated by the two triangles. The BAZ occurs where the water, nutrients, and air flow coincide with the contaminated soil. SOURCE: NRC, 1993.

for permeable soils or by injection through infiltration trenches, galleries, or dry wells for less permeable soils. The infiltration rate must be carefully controlled to preclude soil "flooding," which can decrease the soil's air permeability and leach contaminants to the water table.

When inorganic nutrients or other stimulants are required, they generally are added in soluble form (e.g., NH_4Cl, KNO_3, and NaH_2PO_4) in the infiltrating water. In some cases, nutrients can be added as gases, such as NH_3, N_2O, or triethylphosphate. Addition of methane can be employed to stimulate co-metabolic degradation of TCE. In general, gaseous additives are applied through trenches or wells located in line with the airflow routes.

Water-Circulation Systems When the contamination is partly or totally within the saturated zone, stimulatory materials can be applied by water circulation. Figure 15.3 illustrates the engineered bioremediation of an LNAPL that is above and below the water table. In this case, H_2O_2 is used as a dissolved source of oxygen substantially greater than can be achieved by air saturation. The figure shows a vertical well and a horizontal infiltration gallery; in most cases, one or the other approach is used to add the circulating water and stimulants.

A key to using water circulation is to recover all the circulating water. Recovery systems normally involve a series of extraction wells designed to extract more water than is injected. In this way, there is no export of contaminated groundwater from the site. The extracted water commonly is treated and recycled. Aboveground treatment can include air stripping (shown in Figure 15.3), biological treatment, activated carbon absorption, or a combination. If the extracted water is not

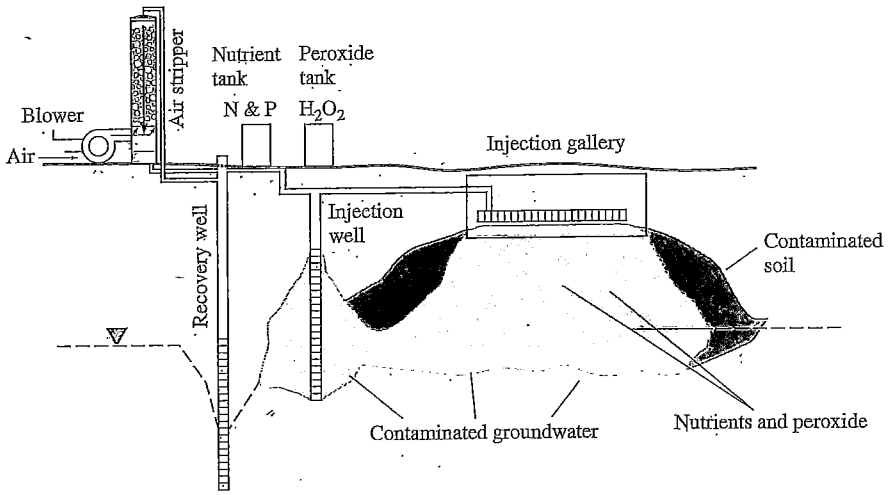

Figure 15.3 Schematic of a water-circulation system with H_2O_2 as an O_2 source. The BAZ is present where the water, nutrients, and oxygen intersect with contaminants dissolved from the contaminated soil. SOURCE: NRC, 1993.

recycled, drinking water or uncontaminated groundwater is amended and used for injection.

One problem that can occur as part of a water-circulation system is clogging near well screens and infiltration galleries (Staps, 1990; Wetzel et al., 1986). Localized growth of bacteria, sometimes coupled with chemical precipitation or gas evolution, reduces the soil's hydraulic conductivity (Rittmann et al., 1994). This can lead to decreased input rates and/or short-circuiting around clogged areas. Efforts to minimize the adverse impact of clogging include pulsing of various substrates and nutrients to "spread out" the BAZ, reduction of substrate loading, back-flushing to dislodge accumulated solids, periodic application of disinfectants (chlorine or additional H_2O_2) or acids, and adjustment of pH and inorganic ions to preclude precipitation.

Air Sparging Originally developed in Europe, air sparging has become a popular means of engineered bioremediation in North America for strictly aerobic biodegradation. Injection of compressed air directly into the contaminated subsurface (Figure 15.4) is an efficient way to deliver oxygen to the BAZ. In addition, air sparging can strip volatile contaminants from the saturated zone into the unsaturated zone and a vapor-capture system. Air sparging is not effective when low-permeability geological zones trap or divert the gas flow.

Nutrients and other amendments can be added from injection wells or an infiltration gallery, as shown in Figure 15.4. Similar to bioventing, some nutrients can be added in a gaseous state. In most cases, a gas-recovery system is needed to capture volatile components and prevent off-site contaminant transport in the gas phase.

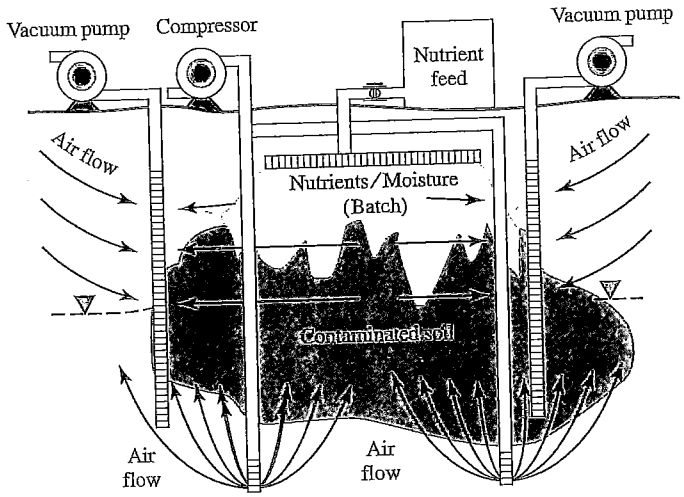

Figure 15.4 An air-sparging system for aerobic treatment of contamination below the water table. The BAZ occurs where the air flow contacts contaminated water. SOURCE: NRC, 1993.

15.5.3 INTRINSIC IN SITU BIOREMEDIATION AND NATURAL ATTENUATION

Intrinsic bioremediation relies on the intrinsic (i.e., naturally occurring) supplies of electron acceptors, nutrients, and other necessary materials to develop a BAZ and prevent the migration of contaminants away from the source. Although intrinsic bioremediation does not accelerate the rate of source cleanup, it can be an effective form of biological containment. Intrinsic bioremediation involves no engineered measures to increase the supply rates of oxygen, nutrients, or other stimulants. On the other hand, intrinsic bioremediation requires careful site characterization in terms of the extent and type of contaminant source and plume, the presence of microorganisms capable of degrading any mobile contaminants, and the intrinsic supply rates of electron acceptor and other required materials. A long-term monitoring program is essential to ensure that the intrinsic biological activity remains effective at preventing contaminant migration. The principles of evaluating bioremediation are given later in this chapter.

Intrinsic in situ bioremediation is best applied in situations in which the groundwater contains naturally high concentrations of electron acceptors and adequate concentrations of nutrients, consistent groundwater levels and flow velocity, and the presence of carbonate materials to buffer pH changes (NRC, 1993). Intrinsic bioremediation can be used alone or in concert with an engineered bioremediation or other technology. Because intrinsic bioremediation depends on naturally occurring supply rates, heavy source contamination can overwhelm the intrinsic supply rates. In such

a case, an engineered technology can be used to reduce the size of the contaminant source until the rate of contaminant dissolution no longer exceeds the intrinsic supply rates.

Intrinsic bioremediation sometimes is confused with *natural attenuation*, which is defined as the reduction of contaminant concentrations due to naturally occurring processes of biodegradation, sorption, advection, dilution, dispersion, and chemical reaction. Intrinsic bioremediation is a more stringent subset of natural attenuation in which microbial transformation reactions are responsible for the decrease in concentration. The National Research Council (NRC, 2000) issued comprehensive guidance for natural attenuation. In principle, natural attenuation can minimize risks from a wide range of organic and inorganic contaminants (NRC, 2000). To date, natural attenuation has been proven to work reliably mainly for BTEX.

15.5.4 IN SITU BIOBARRIERS

A hybrid bioremediation technology is the in situ biobarrier, which is depicted schematically in Figure 15.5. Unlike the previous in situ methods, a biobarrier addresses mobile contaminants already being transported in a plume. The biobarrier is a containment method that prevents further transport of a plume. However, the biobarrier is an engineered system in which a BAZ is created in the path of the plume through injection of electron donor, electron acceptor, and/or nutrients. Hydraulic or physical controls on groundwater movement may be required to make certain that the plume passes through the BAZ. It seems reasonable that several different BAZs could be created sequentially by injection of different electron donors and acceptors. The Moffett Field experiment (Roberts, Hopkins, Mackay, and Semprini, 1990; Semprini, Roberts, Hopkins, and McCarty, 1990) is a small-scale example of creating a specialized BAZ by injecting methane (the carbon source and electron donor) and oxygen (the electron acceptor); cometabolic biodegradation of TCE was demonstrated.

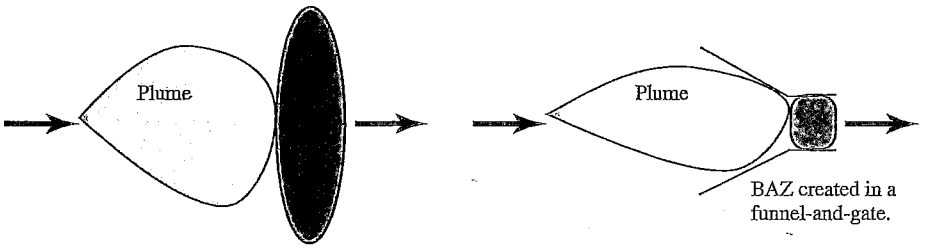

Figure 15.5 Schematic illustration of an in situ biobarrier that contains a mobile contaminant plume by intercepting it with a BAZ. SOURCE: Rittmann et al., 1994.

The materials needed to create a biobarrier can be injected in several ways. The most obvious way is to add them in liquid form through injection wells or trenches. An alternative to simple injection is the recirculating well, in which the injected materials and the plume water are mixed in and around wells that circulate the water between well screens placed at two different levels on the well casing. Full-scale implementation of this technology for cometabolism of TCE was conducted at Edwards Air Force Base, California (McCarty et al., 1998). Slow-release chemical sources of O_2 and H_2 can be placed in the flow path of the plume via wells or trenches sunk to the level of the plume. Iron filings also have been used to create an in situ barrier. The oxidation of the elemental iron can bring about reductive dechlorination of solvents, but it also may spur microbiologically catalyzed reductions.

15.5.5 Ex Situ Bioremediation

Contaminated soils can be excavated and treated in aboveground, or ex situ, treatment systems. Aboveground bioremediation for contaminated solids include slurry reactors, composting, and land farming. Ex situ bioremediation is most applicable for small, heavily contaminated sources and when a rapid site cleanup is desired. Excavation incurs major costs and potentially increases exposure to workers and those who reside nearby.

Slurry reactors contain (typically) 5 percent solids and are vigorously agitated for mixing and aeration. They are advantageous for maximizing the rate of biodegradation. This rate increase is brought about by increased mass-transport rates due to agitation and the ease of adding oxygen, nutrients, and surface-active agents that emulsify NAPLs. The disadvantages of slurry systems are the added costs for capital, operation, and dewatering the decontaminated solids. Slurry reactors tend to be operated in a batch mode, but continuous feeding is possible.

Composting is an aerobic biodegradation scheme operated in a "solid state" format. Solid state means that the compost material behaves as a porous solid with a moisture content of 50 to 60 percent and through which air can be drawn to supply O_2 and to remove evaporated H_2O. Due to the high concentration of biodegradable organic material and the low moisture content of the compost mixture, the temperature rises to 60–70 °C during the time of peak biodegradation rates. In most cases, the air supply is determined by temperature considerations. If the temperature is too hot, more air circulation is needed to drive evaporative cooling. In parallel to control of air circulation, the moisture content must be controlled within the optimal range, 50–60 percent. Too much moisture prevents air circulation and slows microbial reactions. Too little moisture initially allows the temperature to rise above the optimal range; the excess temperature and concomitant desiccation of compost materials arrest biodegradation reactions.

Land farming is a simple, "low-tech" form of ex situ bioremediation. Contaminated solids are mixed into the surface layer of topsoil. Nutrients and moisture can be added initially and throughout the treatment period to optimize conditions for microbial growth. Land farming is most effective for solids contaminated by organic

materials susceptible to aerobic biodegradation and where large tracts of land can be devoted to bioremediation.

15.5.6 PHYTOREMEDIATION

A new and rapidly developing form of bioremediation is *phytoremediation,* which uses green plants and their associated biota to destroy, remove, contain, or otherwise detoxify environmental contaminants (Cunningham, Anderson, Schwab, and Hsu, 1996). Four types of destruction and removal reactions can occur in phytoremediation. Definitions of the four pathways are:

- *Phytovolatilization* is an enhancement of the volatilization process from the soil or through the plant's roots or shoots. Enhanced volatilization can occur via plant transpiration of volatile compounds or transformation of contaminants to more volatile forms.
- *Phytodegradation* involves uptake by the plant and subsequent metabolism by plant enzymes to form benign products.
- *Phytoextraction* involves uptake by the plant and absorption of the contaminant into plant tissue, which subsequently is harvested. Hydrophobic contaminants are most susceptible to photoextraction.
- *Rhizo(sphere)degradation* to benign products is catalyzed by plant enzymes excreted by the roots or by the microorganisms found in the rhizosphere.

In addition, phytoremediation can sequester contaminants, a process called *phytostabilization* (Cunningham et al., 1996). The benefit of phytostabilization is that it makes the contaminants less bioavailable to humans and other receptors. Three forms of phytostabilization can be identified:

- *Humification* incorporates contaminants into the soil organic matter, or humus, through binding (polymerization) reactions of plant and microbial enzymes.
- *Lignification* incorporates the contaminant into plant cell-wall constituents.
- *Aging* slowly binds the compounds into the soil mineral fraction.

The distinctions among the various stabilization, destruction, and removal mechanisms often are not sharp. For example, rhizosphere peroxidases can catalyze oxidation reactions that lead to contaminant mineralization (degradation) or to polymerization (humificaction).

Phytoremediation is still in its early stages of research and development. Nonetheless, it has promise for the decontamination of petroleum hydrocarbons, a range of chlorinated aliphatics and aromatics (including PCBs), and pesticides (Cunningham et al., 1996). Phytoextraction of heavy metals is another promising application (Moffat, 1995).

Another aspect of phytoremediation is hydraulic control. During their growing season, plants transpire large volumes of water to the atmosphere. This transpiration can stop the downward flow of precipitation from the vadose zone to the saturated zone. It also can "pump" water from the top of the saturated zone to the atmosphere.

Transpiration is being exploited as a means to stop or slow the migration of contaminants that are in the vadose zone or the boundary between the vadose and saturated zones. Of course, this pumping action only occurs when the plants are active in photosynthesis. For example, deciduous trees are ineffective in the winter.

15.5.7 BIOREMEDIATION OF GAS-PHASE VOCs

Gases contaminated with volatile organic compounds can be generated in numerous ways:

- Bioventing and air sparging, as discussed above
- Vapor extraction of contaminated soils (NRC, 1994)
- Storage tanks or production facilities for volatile organic products, such as gasoline, petroleum, and solvents.
- Off gases from wastewater treatment processes
- Off gases from anaerobic treatment processes
- Off gases from sewers or preliminary treatment of sewage

Volatile organic compounds (VOCs) are organic chemicals that have a boiling point $\leq$ 100 °C and/or a vapor pressure $\geq$ 1 mm Hg at 25 °C. They include alkane gases (e.g., methane), alcohols (e.g., methanol), low-molecular-weight petroleum hydrocarbons (e.g., benzene and toluene), halogenated aromatics (e.g., chlorobenzenes), and halogenated aliphatic solvents (e.g., 1,1,1-TCA and TCE). These VOCs are of particular environmental concern because they are very mobile, may affect human senses through odor, may exert a narcotic effect, and may be toxic and carcinogenic. Some VOCs also react photochemically to form ground-level ozone, a key component of smog, while others are greenhouse gases. For these reasons, emissions of VOCs to the atmosphere are becoming a greater concern to regulatory agencies, industry, and the general public.

Biodegradation of VOCs is among a suite of technologies that can be used to treat gases containing VOCs (Moretti and Mukhopadhyay, 1993). Low capital and operating costs are the main advantages of biofiltration, the main type of biological treatment of gases. For example, Bohn (1992) found that the capital costs of a typical biofiltration system were only about 6 percent of those for incineration, 13 percent of those for ozone oxidation, and 40 percent of those for activated-carbon adsorption.

Ottengraf (1987) described the biological systems commonly being used or tested in Europe, Japan, and the USA for treatment of gas-phase VOCs, as well as NH_3, H_2S, and semi-volatile odor compounds. *Bioscrubbers* countercurrently contact the gas stream with circulating liquid in a spray column. The bacteria are mainly dispersed in the circulating liquid. In *trickling filters* and *biofilters,* the microorganisms are immobilized on a carrier or packing medium. In most cases, the water flow is downward by gravity, while the gas flow is countercurrent, or upward. Trickling filters are distinguished from biofilters by the amount of water applied. The trickling filter has a larger hydraulic loading so that the aqueous phase continuously flows downward. Because the hydraulic loading is less for biofilters, the water phase is essentially stationary.

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Biological treatment of contaminated gas phases also can be accomplished by passing the gas through compost, peat, or soil beds (Ottengraf, Meeters, van den Oever, and Rozema, 1986; Wilson, Pogue, and Canter, 1988). These systems are very simple, although process control may be limited. More recent developments include using three-phase fluidized-bed biofilm reactors to create highly compact biofilm systems well suited to industrial settings.

Except for bioscrubbers, all of the gas-treatment systems are of the biofilm type. Therefore, the principles and design approaches provided in Chapters 4 and 8 are directly applicable here. The main difference is that the contaminants enter in a gas stream, which must be included in the mass balances. Because most of the contaminants are hazardous organic compounds, the information in Chapter 14 is of the utmost relevance.

15.6 EVALUATING BIOREMEDIATION

One of the factors that limit the widespread application of bioremediation is that its "success" has been difficult to "prove" in the field. Regulators, site owners, and the public are wary of a technology that requires a sophisticated appreciation for microbiology and how it connects to the hydrogeology and chemistry of contaminated soils. When measures for the technology's success are neither obvious nor agreed upon, the wary decision maker is likely to seek other solutions, including expensive alternatives, such as pump-and-treat or incineration.

The development and acceptance of evaluation protocols is a slow process for the following reasons:

- The definition of success varies among the several parties involved. Regulators generally define success only in terms of meeting some compliance level for contaminant concentration in the water or soil. Buyers of bioremediation are acutely interested in cost-effectiveness. The public generally wants assurance that they are not being exposed to risk. Researchers and developers of bioremediation wish to demonstrate the cause-and-effect nature of the process. This means that agreement on evaluation protocols requires sustained communication among the many parties who are stakeholders and/or need to contribute key technical judgements: regulators, buyers, the public, engineers, microbiologists, hydrogeologists, chemists, and others.
- Many different measures of success can be put forward, but no one measure is universally applicable for a type of contamination, soil conditions, or bioremediation approach. Expert and site-specific judgment always will play a role.
- Contaminated soils are inherently heterogeneous. In situ technologies amplify the importance of heterogeneity, since sampling is difficult and expensive, while microorganisms often are highly localized. It is well known that finding residual NAPL contamination is extremely difficult. Because the microorganisms often accumulate on solids close to the trapped contaminants, they also are difficult to find.

Although evaluating bioremediation is challenging and not easily standardized, it can be accomplished if an evaluation program is properly designed (NRC, 1993, 2000). The key to an evaluation program is that the measures of "success" must directly link microbial activity to the observed loss of contaminant. To obtain this linkage, the NRC (1993) recommends an evaluation strategy based on three kinds of evidence:

1. documented loss of contaminants from the site
2. laboratory assays showing that microorganisms at the site have the *potential* to transform the contaminants under the expected site conditions
3. one or more pieces of evidence showing that the biodegradation potential is *actually realized* in the field

The third type of evidence is the most crucial and the most difficult to obtain. It links the loss of contaminants in the soil and water with the potential for them to be biodegraded. A wide range of measurement approaches is available, and the NRC (1993) discusses their scientific bases. Table 15.11 briefly summarizes some of the techniques and what they indicate. In all cases, the measurement techniques should be used to (1) show that characteristics of the site's chemistry or microbial population change in ways one would predict if bioremediation were occurring and (2) correlate these chemical and microbial changes with documented contaminant losses.

One key distinction is between techniques that can provide principal evidence versus those that provide only confirmatory evidence (Rittmann et al., 1994). Principal evidence should be equally capable of proving the success or failure of bioremediation. Among the various techniques in Table 15.11, good examples of principal evidence include stoichiometric consumption of electron acceptors, formation of inorganic carbon that originated in organic carbon, and increased degradation rates over time. Quantification of consumption or formation rates is of great importance. The mere demonstration that a reaction occurs is not always principal evidence; instead, the rate must commensurate with the loss of contamination.

Confirmatory evidence, on the other hand, usually only can support success, but its absence does not prove failure. Good examples of confirmatory evidence include increases in the populations of bacteria or protozoa (they are too hard to find to be principal), detection of intermediary metabolites (they can be degraded themselves), and an increase to the ratio of nondegradable to degradable components (other mechanisms also act differentially).

The complexities of the contaminated setting and the difficulties in obtaining good samples make it rare for one type of measurement to give unequivocal proof. Therefore, the three-part strategy relies upon building a consistent, logical case from convergent lines of independent evidence (NRC, 1993). A wide range of principal and confirmatory techniques is needed to create a strong case for proving (or disproving) the success of a bioremediation project.

Since the NRC's 1993 report, a number of public and private organizations have issued protocols for evaluating intrinsic bioremediation and natural attenuation. The protocols vary widely in terms of scope, detail, and adequacy. A critical evaluation of these protocols, carried out recently by a committee of the NRC (2000), provides

Table 15.11 Summary of techniques used to demonstrate biodegradation in the field

Technique	Indication of
Direct Measurements of Field Samples	
Number of bacteria	An increase in the population of contaminant-degrading bacteria over background conditions
Number of protozoa	An increase in the population of predators of the contaminant-degrading bacteria
Rate of bacterial activity in laboratory microcosms	Potential rates of biodegradation
Bacterial adaptation	An increase in the rates of biodegradation since bioremediation began
Inorganic carbon concentration	Formation of inorganic carbon by oxidation of the organic contaminant
Carbon isotope ratio	Inorganic carbon originating from organic contamination
Electron-acceptor concentration	A decrease in the electron acceptors used during contaminant oxidation
By-products of anaerobic activity	Formation of products generated when electron acceptors other than O_2 are used
Intermediary metabolites	Breakdown products of complex organic contaminants
Ratio of nondegradable to degradable components	Relative loss of biodegradable components
Experiments run in the field	
Stimulating bacteria within subsites	An increase in microbial activity with stimulation used in engineered bioremediation
Measuring electron-acceptor uptake rate	In situ rate of metabolism
Monitoring a conservative tracer	Contaminant losses due to abiotic mechanisms
Labeling contaminants	The fate of carbon contained in organic contaminants
Modeling Experiments	
Modeling abiotic mass loss	Potential losses through abiotic mechanisms
Direct modeling	In situ biodegradation rates

SOURCE: Rittmann et al., 1994.

guidance on what should be included in an evaluation scheme. The foundation of the NRC's 2000 guidance is that the monitoring and evaluation program should document the presence of "footprints," which are the products of the biological reactions capable of destroying the contaminants. Footprints include:

- Loss of electron acceptors in proportion to loss of a contaminant that is oxidized
- Increases in inorganic carbon in proportion to the loss or organic contaminants that are mineralized

- Loss of an electron-donor substrate in parallel to the loss of a contaminant that is reduced, such as by reductive dechlorination
- Release of the chloride ion from organic chemicals that are dechlorinated
- Increases or decreases of alkalinity in proportion to reactions that release base or release acid, respectively

In some cases, confounding reactions or high background levels make it impossible to detect all the relevant footprints. Nevertheless, the detection of several footprints at levels commensurate to the loss of contaminant is necessary to ensure that natural attenuation is reliably protecting public health and the environment.

The level of effort to monitor a site depends on the likelihood that a natural-attenuation mechanism is able to destroy the contaminant in a given setting and the complexity of the setting. Reduced likelihood of destruction and/or a more complex site demands increases to the number, extent, and frequency of sampling, as well as more sophisticated evaluation of the data. These issues are discussed by NRC (2000).

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15.8 PROBLEMS

15.1. What factors lead to recalcitrance of an organic molecule discharged to soil?

15.2. You are considering in situ biodegradation of the chlorinated solvent trichloroethene or TCE. Indicate whether aerobic or anaerobic treatment or both have good potential for degradation of this compound, and indicate the biological processes involved in the treatment or treatments with good potential and the intermediate compounds that are likely to be formed.

15.3. Three separate industries are potentially responsible for contamination of a municipal well supply with chlorinated aliphatic hydrocarbons (CAHs). Industry records indicate that Industry A was a large user of carbon tetrachloride (CT), Industry B was a large user of tetrachloroethene (PCE), and Industry C was a large user of 1,1,1-trichloroethane (TCA). Are far as can be determined, each industry used only one chlorinated solvent. The only CAHs found present in the municipal well supply are vinyl chloride, 1,1-dichloroethene, and 1,1-dichloroethane. Since none of the industries used these compounds, they all claim they are innocent. You are called in by the regulatory authorities as an expert witness in this case to help shed some light on the possible source or sources of these contaminants.

(*a*) Does the evidence suggest the identified industries are innocent as claimed and that some unidentified industry (D) is responsible, or does it support the potential that one or all of the identified industries may be responsible?

(*b*) Who is the most likely culprit?

Give technically sound reasons for your judgments.

15.4. Describe biological transformations known to occur that might be used for in situ biodegradation of TCE? What chemicals would you need to add if not already present in order to bring about such TCE transformations?

15.5. You have been called in to consider *in situ* bioremediation of an earthen heavy equipment storage yard, the soil of which has been contaminated with oil leaking from heavy trucks, bulldozers, and other equipment that had been stored there for years. The average concentration of organic hydrocarbons (HC) in the upper 0.3 m of soil, where the contamination resides, has been measured to be 0.1 kg HC/kg soil. Describe the treatment you might consider giving to the contaminated soil, and quantify the amounts of materials, if any, that you might apply to help speed biodegradation.

15.6. You are called upon to investigate the sources of contamination of an aquifer and to recommend possible biological methods for degrading the contaminants found. For your investigation, you have found high concentrations (low mg/l range) of 1,1-dichloroethene (1,1-DCE); 1,2-*cis* dichloroethene (1,2-c-DCE);

1,2-*trans*-dichloroethene (1,2-t-DCE); and 1,1-dichloroethane (1,1-DCA); together with somewhat lower concentrations of 1,1,1-trichloroethane (TCA), trichloroethene (TCE), and vinyl chloride (VC).

(*a*) What might you expect to be the original contaminants that entered the subsurface system studied? Why?

(*b*) What different biological processes might bring about transformation of the contaminants? For each indicate respective electron donors and acceptors that would be required for the transformation.

(*c*) Considering the above one or more biological processes, indicate the possible advantages and disadvantages of each.

(*d*) Draw a pictorial flow sheet of a possible treatment scheme that you might consider for treating the above groundwater by one of the above biological processes. Show a profile of the surface and subsurface system, locations of points of injection or extraction, possible aboveground or subsurface reactors, locations of chemical injection, etc.

15.7. Compute the retardation factor for toluene ($\log K_{ow} = 2.7$), vinyl chloride ($\log K_{ow} = 0.6$), and phenanthrene ($\log K_{ow} = 4.6$) for a sandy soil ($f_{oc} = 0.001$) and a peat soil ($f_{oc} = 0.1$) (Six answers).

15.8. For the six conditions of problem 15.7, determine what fraction of the solute's mass is present in the water phase when the porosity is 0.3 and the bulk soil density is 2 kg/l (Six answers).

15.9. Which is the better engineered bioremediation technology among air sparging, bioventing, and addition of glucose for these scenarios:

(*a*) Petroleum hydrocarbons strictly in the vadose zone

(*b*) Petroleum hydrocarbons with residual in the saturated zone

(*c*) TCE as a DNAPL.

15.10. Which of these scenarios is likely to be successful for intrinsic bioremediation?

(*a*) A leaking gasoline storage tank in a sandy soil

(*b*) A leaking storage tank of TCE in an otherwise clean aquifer

(*c*) A leaking storage tank of TCE next to a leaking storage tank of gasoline

(*d*) A deposit of polynuclear aromatic hydrocarbons in a sandy aquifer.

15.11. Compute the equilibrium concentration of toluene in groundwater if the water is in contact with gasoline having 6 percent toluene.

15.12. 18.4 mg/l of toluene is in a groundwater plume. Compute how much of the following would be necessary for full mineralization of the toluene if each reaction were the only reaction responsible for accepting the electrons from toluene. You may ignore biomass growth. Express the result as a concentration in the groundwater (mg/l).

(*a*) O_2 consumption in aerobic respiration, mgO_2/l

(*b*) SO_4^{2-} consumption in sulfate reduction to sulfides, mgS/l

(*c*) NO_3^- consumption by denitrification to N_2, mgN/l

(*d*) $Fe(OH)_3$ reduction to Fe^{2+}, mgFe/l

(*e*) CH_4 generation in methanogenesis, $mgCH_4/l$