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5 *IN SITU* BIOREMEDIATION

5.1 INTRODUCTION

Biological processes, which take place in the natural environment, can modify organic contaminant molecules at the spill location or during their transport in the subsurface. Such biological transformations, which involve enzymes as catalysts, frequently bring about extensive modification in the structure and toxicological properties of the contaminants. These biotic processes may result in the complete conversion of the organic molecule to innocuous inorganic end products, cause major changes that result in new organic products, or occasionally lead to only minor modifications. The available body of information suggests that the major agents causing the biological transformations in soil, sediment, surface water, and groundwater are the indigenous microorganisms that inhabit these environments.

Biodegradation can be defined as the microbially catalyzed reduction in complexity of chemicals. In the case of organic compounds, biodegradation frequently, although not necessarily, leads to the conversion of much of the carbon, nitrogen, phosphorus, sulfur, and other elements in the original compound to inorganic end products. Such a conversion of an organic substrate to inorganic end products is known as mineralization. Thus, in the mineralization of organic C, N, P, S, or other elements, CO_2 or inorganic forms of N, P, S, or other elements are released by the organisms and enter the surrounding environment. Few nonbiological reactions in nature bring about comparable changes.

Natural communities of microorganisms present in the subsurface have an amazing physiological versatility. Microorganisms can carry out biodegradation in many different types of habitats and environments, both under aerobic and anaerobic conditions. Communities of bacteria and fungi can degrade a multitude of synthetic compounds and probably every natural product.

In situ bioremediation is the application of biological treatment to the cleanup of hazardous chemicals present in the subsurface. The optimization and control of microbial transformations of organic contaminants require the integration of many scientific and engineering disciplines.

Hazardous compounds persist in the subsurface because environmental conditions are not appropriate for the microbial activity that results in biochemical degradation. The optimization of environmental conditions is achieved by understanding the biological principles under which these compounds are degraded, and the effect of environmental conditions on both the responsible microorganisms and their metabolic reactions. The “biodegradation triangle” (Figure 5.1) for understanding the microbial degradation of any natural or synthetic organic compound consists of knowledge of the microbial community, environmental conditions, and structure and physicochemical characteristics of the organic compound to be degraded.

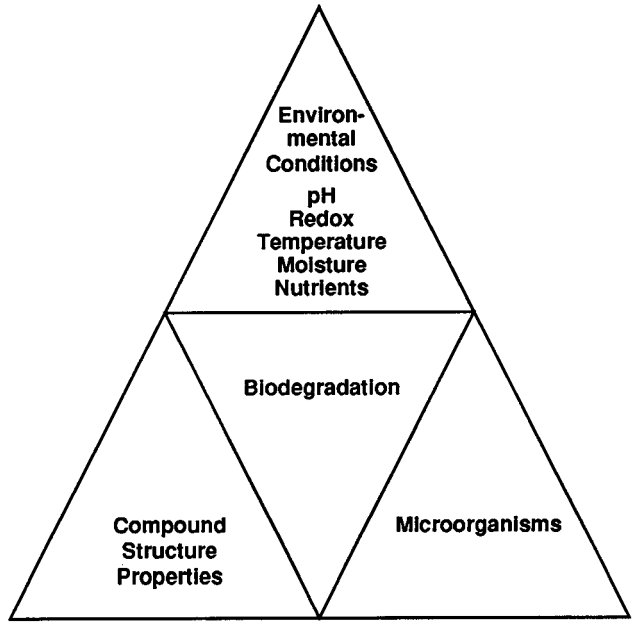


Figure 5.1 Biodegradation triangle.

5.2 MICROBIAL METABOLISM

During the process of *in situ* bioremediation, microorganisms use the organic contaminants for their growth. In addition, compounds providing the major nutrients such as nitrogen, phosphorus, and minor nutrients such as sulfur and trace elements are also required for their growth. In most cases, an organic compound that represents a carbon and energy source is transformed by the metabolic pathways that are characteristic of heterotrophic microorganisms. It should be stressed, however, that an organic compound need not necessarily be a substrate for growth in order for it to be metabolized by microorganisms. Two categories of transformations exist. In the first, biodegradation provides carbon and energy to support growth, and the process, therefore, is growth-linked. In the second, biodegradation is not linked to multiplication, but to obtaining the carbon for respiration in order for the cells to maintain their viability. This maintenance metabolism may take place only when the organic carbon concentrations are very low. Cometabolic transformations also fall into the second category.

It has been observed that the number of microbial cells or the biomass of the species acting on the compound of interest increases as degradation proceeds.[1] During a typical growth-linked mineralization brought about by bacteria, the cells use some of the energy and carbon of the organic substrate to make new cells, and this increasingly larger population causes increasingly rapid mineralization.

Microorganisms need nitrogen, phosphorus, and sulfur, and a variety of trace nutrients other than carbon. These requirements should be satisfied as the responsible species degrade the compound of interest. For heterotrophic microorganisms in most natural systems, usually sufficient amounts of N, P, S, and other trace nutrients are present to satisfy the microbial demand. Because carbon is limiting and because it is the element for which there is intense competition, a species with the unique ability to grow on synthetic molecules has a selective advantage.

Prior to the degradation of many organic compounds, a period is observed in which no degradation of the chemical is evident. This time interval is known as the *acclimatization*

period or, sometimes, as adaptation or lag period. The length of the acclimatization period varies and may be less than 1 h or many months. The duration of acclimatization depends upon the chemical structure, subsurface biogeochemical environmental conditions, and concentration of the compound. Once the indigenous population of microorganisms has become acclimatized to the presence and degradation of a chemical and the activity becomes marked, the microbial community will retain its higher level of activity for some time. Acclimatization of a microbial population to one substrate frequently results in the simultaneous acclimatization to some, but not all, structurally related molecules.

5.2.1 Metabolism Modes

The design of bioremediation processes requires determination of the desired degradation reactions to which the target compounds will be subjected. This involves selecting the metabolism mode that will occur in the process. The metabolism modes are broadly classified as aerobic and anaerobic. Aerobic transformations occur in the presence of molecular oxygen, with molecular oxygen serving as the electron acceptor. This form of metabolism is known as *aerobic respiration*. Anaerobic reactions occur only in the absence of molecular oxygen and the reactions are subdivided into *anaerobic respiration*, *fermentation*, and *methane fermentation*.

Microorganisms have developed a wide variety of respiration systems. These can be characterized by the nature of the reductant and oxidant. In all cases of aerobic respiration, the electron acceptor is molecular oxygen. Anaerobic respiration uses an oxidized inorganic or organic compound other than oxygen as the electron acceptor. The respiration of organic substrates by bacteria is, in most cases, very similar. The substrates are oxidized to CO_2 and H_2O.

Fermentation is the simplest of the three principal modes of energy yielding metabolism. During fermentation, organic compounds serve as both electron donors and electron acceptors. Fermentation can proceed only under strictly anaerobic conditions. The process maintains a strict oxidation-reduction balance. The average oxidation level of the end products is identical to that of the substrate fermented. Thus the substrate yields a mixture of end products, some more oxidized than the substrate and others more reduced. The end products depend on the type of microorganisms but usually include a number of acids, alcohols, ketones, and gases such as CO_2 and CH_4. Table 5.1 summarizes the various microbial metabolic reactions.

Table 5.1 Summary of Metabolism Modes

Reductant electron donor	Oxidant electron acceptor	End products
Aerobic respiration		
Organic substrates (benzene, toluene, phenol)	O_2	CO_2, H_2O
NH_4	O_2	NO_2^-, NO_3^-, H_2O
Fe^{2+}	O_2	Fe^{3+}
S^{2-}	O_2	SO_4^{--}
Anaerobic respiration		
Organic substrates (benzene, toluene, phenol, trichloroethylene)	NO_3^-	N_2, CO_2, H_2O, Cl^-
Organic substrates (benzene, trichloroethylene)	SO_4^{2-}	S^{2-}, H_2O, CO_2, Cl^-
H_2	SO_4^{2-}	S_2^-, H_2O
H_2	CO_2	CH_4, H_2O
Fermentation		
Organic substrates	Organic compounds	Organic compounds CO_2, CH_4

The metabolism modes that utilize nitrate as an electron acceptor (performed by denitrifying and nitrate-reducing organisms), sulfate and thiosulfate as electron acceptors (performed by sulfate-reducing organisms), and CO_2 as an electron acceptor (performed by methanogenic organisms) can be used to biodegrade various organic contaminants. The utilization of chlorinated organic compounds as electron acceptors during anaerobic respiration is a recent observation.

Another important metabolism concept in bioremediation is cometabolism. In a true sense, cometabolism is not metabolism (energy yielding), but fortuitous transformation of a compound. As noted earlier, it was the traditional belief that microorganisms must obtain energy from an organic compound to biodegrade it. The transformation of an organic compound by a microorganism that is unable to use the substrate as a source of energy is termed cometabolism.[1] Enzymes generated by an organism growing at the expense of one substrate also can transform a different substrate that is not associated with that organism's energy production, carbon assimilation, or any other growth processes.

Contaminants that lend themselves to bioremediation by becoming a secondary substrate through cometabolism are only partially transformed. This transformation may or may not result in reducing toxicity. If all toxicity properties of a hazardous compound are removed via biotransformation, this is referred to as detoxification. Detoxification results in inactivation, with the toxicologically active substance being converted to an inactive product. Detoxification does not imply mineralization and may include several processes such as hydrolysis, hydroxylation, dechlorination, and demethylation. Fortunately, the metabolites or transformation products from cometabolism by one organism can typically be used as an energy source by another.

Since cometabolism generally leads to a slow degradation of the substrate, attention has been given to enhancing its rate. The addition of a number of organic compounds into the contaminated zone may promote the rate of cometabolism,[1] but the responses to such additions are not predictable. Addition of mineralizable compounds that are structurally analogous to the compound whose cometabolism is desired is known as *analog enrichment*.[1] The microorganism that grows on the mineralizable compound contains enzymes transforming the analogous molecule by cometabolism.

Another aspect of microbial metabolism is the recognition of preferential substrate degradation. Preferential degradation results in a sequential attack where the higher energy-yielding compounds are degraded first. In a petroleum spill, benzene will be degraded, under aerobic conditions, at a faster rate than naphthalene, and naphthalene will degrade faster than chrysene.

5.3 MICROBIAL REACTIONS AND PATHWAYS

Microbial transformations of organic compounds are frequently described by the terms, degradation, mineralization, detoxification, and activation. Degradation means that the initial substrate no longer exists. Mineralization refers to the complete conversion of the organic structure to inorganic forms such as CO_2, H_2O, and Cl^-. Detoxification is the transformation of the compound to some intermediate form that is nontoxic or less toxic. The process of forming toxic end products or intermediate products is known as activation.

Microorganisms are capable of catalyzing a variety of reactions: dechlorination, hydrolysis, cleavage, oxidation, reduction, dehydrogenation, dehydrohalogenation, and substitution.

- *Dechlorination*—the chlorinated compound becomes an electron acceptor; in this process, a chlorine atom is removed and is replaced with a hydrogen atom.
- *Hydrolysis*—frequently conducted outside the microbial cell by exoenzymes. Hydrolysis is simply a cleavage of an organic molecule with the addition of water.

- *Cleavage*—cleaving of a carbon–carbon bond is another important reaction. An organic compound is split or a terminal carbon is cleaved off an organic chain.
- *Oxidation*—breakdown of organic compounds using an electrophilic form of oxygen.
- *Reduction*—breakdown of organic compounds by a nucleophilic form of hydrogen or by direct electron delivery.
- *Dehydrogenation*—an oxidation–reduction reaction that results in the loss of two electrons and two protons, resulting in the loss of two hydrogen atoms.
- *Dehydrohalogenation*—similar to dechlorination, results in the loss of a hydrogen and chlorine atom from the organic compound.
- *Substitution*—these reactions involve replacing one atom with another.

Examples of these reactions are shown in Table 5.2.

Table 5.2 Microbially Catalyzed Reactions

Reaction	Example
• Dehalogenation	$Cl_2C = CHCl + H^+ \rightarrow ClHC = CHCl + Cl^-$
• Hydrolysis	$RCO - OR' + H_2O \rightarrow RCOOH + R'OH$
• Cleavage	$RCOOH \rightarrow RH + CO_2$
• Oxidation	$CH_3CHCl_2 + H_2O \rightarrow CH_3\ CCl_2\ OH + 2H^+ + 2e^-$
• Reduction	$CCl_4 + H^+ + 2e^- \rightarrow CHCl_3 + Cl^-$
• Dehydrohalogenation	$CCl_3CH_3 \rightarrow CCl_2CH_2 + HCl$
• Substitution	$CH_3CH_2Br + HS^- \rightarrow CH_3CH_2SH + Br^-$

From McCarty, P. L. and Semprini, L., Groundwater treatment for chlorinated solvents, in *Handbook of Bioremediation*, Norris, R. D., et al., Eds., Lewis Publishers, Boca Raton, FL, 1994. With permission.

5.3.1 Hydrocarbons Degradation

5.3.1.1 Aliphatic Hydrocarbons

Hydrocarbons are compounds containing carbon and hydrogen. Aliphatic hydrocarbons are straight- or branched-chain hydrocarbons of various lengths. The aliphatic hydrocarbons are divided into the families of alkanes, alkenes, alkynes, alcohols, aldehydes, ketones, and acids. There are cyclic aliphatic hydrocarbons which have diverse structures. Typical structures of aliphatic hydrocarbons are shown in Figure 5.2.

The most frequent and earliest application of *in situ* bioremediation has been to remediate hydrocarbons present in the subsurface as a result of petroleum spills. The degradation potential of alkanes is a function of the carbon chain length. Short chains are more difficult to degrade than the longer chains. Soil contains significant populations of microbes that can use select hydrocarbons as sole sources of carbon and energy. Soil populations capable of degrading hydrocarbons have been reported as high as 20% of all soil microbes.[3] Fungi and yeast are also capable of degrading aliphatic hydrocarbons in addition to bacteria.

Bioremediation of aliphatic hydrocarbons should be performed as an aerobic process. Conclusive evidence for anaerobic degradation of aliphatic hydrocarbons, although referenced, is uncertain and, at this stage, too ill-defined.

Aerobic biodegradation of aliphatic hydrocarbons involves the incorporation of molecular oxygen into the hydrocarbon structure. This is performed by oxygenase enzymes. There are two groups of oxygenases: monooxygenases and dioxygenases.[4] The most common pathway of alkane degradation is oxidation at the terminal methyl group. Oxidation proceeds as a sequence to an alcohol to the corresponding fatty acid to a ketone and eventually to carbon dioxide and water.[2] Short-chain hydrocarbons, except methane, are more difficult to degrade. Under aerobic conditions, methane is readily used as the sole carbon source by methanotrophs.

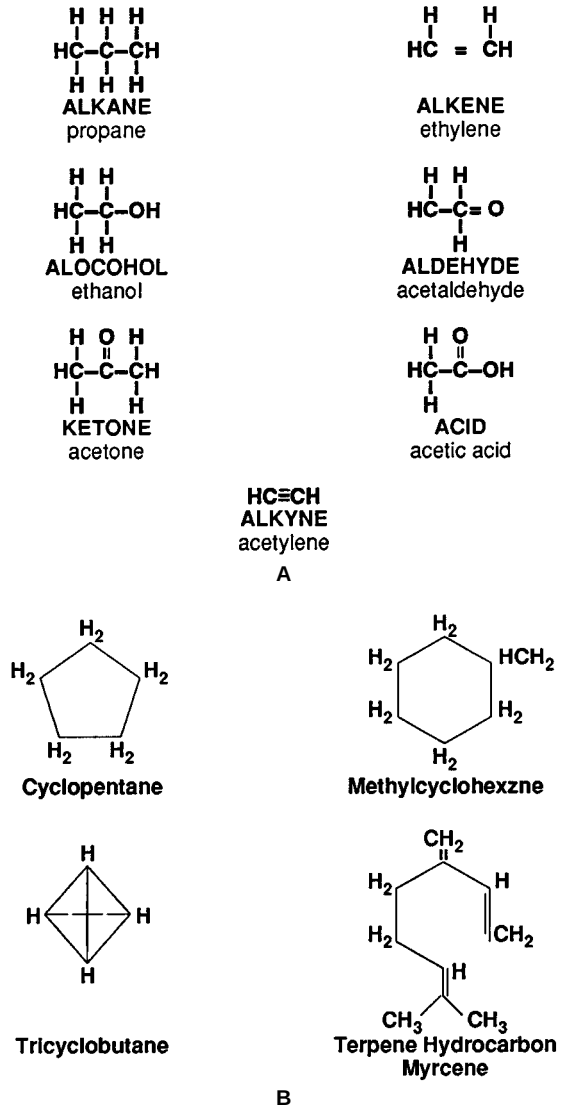


Figure 5.2 Structures of aliphatic hydrocarbons. A. Straight-chain or branched. B. Cyclic.

Alkene degradation, where a C=C double bond is involved, is more varied since microbial attack can occur at either the methyl group or the double bond.[2] Unsaturated straight-chain hydrocarbons are generally less readily degraded than saturated ones. Methyl group oxidation is considered the major degradation pathway during alkene degradation.

Hydrocarbons with branch chains are less susceptible to degradation. Even more resistant to degradation are the quaternary carbon compounds, in which one carbon atom is attached to four other carbon atoms.

Microorganisms capable of degrading cyclic aliphatic hydrocarbons are not as predominant in soils as those for the degradation of aliphatic alkane and alkene hydrocarbons.[2] Hydroxylation is vital to initiate the degradation of cycloalkanes.

5.3.1.2 Aromatic Hydrocarbons

Carbon skeletons that contain the benzene ring as the parent structure are known as aromatic hydrocarbons. They are all ring compounds and have only one free valence bond.

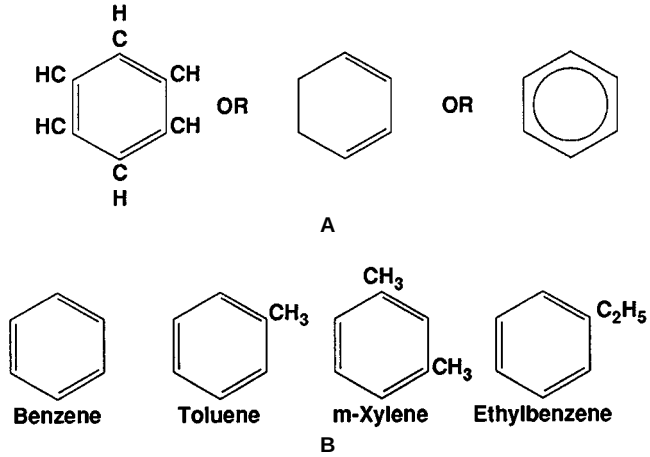


Figure 5.3 Examples of single-ring aromatic compounds. A. Benzene formula and representations. B. BTEX compounds.

The benzene ring is represented by double bonds between alternate carbon atoms. The single ring structures consist of benzene, toluene, ethyl benzene, and the three isomers of xylene (ortho, para, and meta) (Figure 5.3). These are frequently referred to as BTEX compounds and are one of the most heavily regulated group of compounds.

The hydrogens in the aromatic hydrocarbon may be substituted by a variety of different groups: OH, Cl, Br, NO_2, NO, and CN to name a few.

Aromatic compounds can be easily biodegraded, are extremely resistant, or yield undesirable intermediates. These differences depend on the number of rings in the structure, the number of substitutions, and the type and position of substituted groups. Microorganisms capable of aerobically metabolizing single-ring aromatic hydrocarbons are ubiquitous in the subsurface. The degradation is achieved by two alternate modes of oxidation.[2] The first method involves, sequentially: (1) formation of dihydrodiol, (2) formation of alkyl catechol, (3) ring fission of these oxygenated intermediates, (4) formation of either an aldehyde or an acid, and (5) eventual formation of CO_2 and H_2O. In principle, the degradation follows the dioxygenase route, which means the insertion of two oxygen groups.[5] The molecule is transformed to a smaller size, gradually "breaking off" CO_2 units. The second mechanism for degradation of aromatic compounds is oxidation of any alkyl substitutes in the ring.[2]

The stoichiometric equation of benzene degradation in the presence of O_2 is shown in equation (5.1) below. Based on this equation, the mass ratio of O_2 to benzene is 3.1:1; thus, 0.32 mg/l of benzene will be degraded per 1 mg/l of O_2 consumed by the microorganisms during aerobic biodegradation.

$$7.5O_2 + C_6H_6 \rightarrow 6CO_2 + 3H_2O\,. \tag{5.1}$$

Aromatic hydrocarbons can be transformed under various anaerobic conditions such as denitrifying, manganese reducing, iron reducing, sulfate reducing, and methanogenic conditions. At any given location, the benzene biodegradation sequence will depend on the availability of electron acceptors and the redox potential of the environment. This sequence is shown in Table 5.3.

Under denitrifying conditions, degradation of monoaromatic compounds has been demonstrated in a number of systems.[7] The stoichiometry of the denitrification reaction of benzene, assuming no cell growth with NO_3^- reduced completely to N_2 and benzene oxidized completely to CO_2 is

$$C_6H_6 + 6H^+ + 6NO_3^- \rightarrow 6CO_2 + 3N_2 + 6H_2O\,. \tag{5.2}$$

Table 5.3 Benzene Biodegradation under Various Electron Acceptor and Redox Conditions

Redox potential	Reaction type	Electron acceptors	Byproducts
>300 mv	Aerobic	O_2	CO_2, H_2O
	Denitrification	NO_3^-	NO_2^-, N_2, CO_2, H_2O
↓	Valence reduction	Mn(IV)	Mn(II), CO_2, H_2O
	Valence reduction	Fe(III)	Fe(II), CO_2, H^+
	Sulfate reduction	SO_4^{2-}	S_2^-, CO_2, H_2O
–300 mv	Methanogenesis	CO_2	CO_2, CH_4

Iron (Fe(III)) reducing conditions will also facilitate the degradation of monoaromatic hydrocarbons. Relative to other anaerobic processes, Fe(III) reduction has a very unfavorable substrate to electron acceptor ratio. The stoichiometric equation for the degradation of benzene under Fe(III) reducing conditions is

$$C_6H_6 + 30Fe^{3+} + 12H_2O \rightarrow 6CO_2 + 30H^+ + 30Fe^{2+}. \quad (5.3)$$

Biodegradation using sulfate as the electron acceptor involves oxidation of aromatic hydrocarbons by sulfate-reducing microorganisms coupled with reduction of sulfate to hydrogen sulfide.[7] For benzene, the stoichiometry of this reaction, assuming no cell growth is

$$C_6H_6 + 3.75SO_4^{2-} + 7.5H^+ \rightarrow 6CO_2 + 3.75H_2S + 3H_2O. \quad (5.4)$$

Under methanogenic (fermentative) conditions, several aromatic hydrocarbon compounds, including benzene, have been shown to transform into CO_2 and methane.[7] Assuming no cell growth, the stoichiometry for the transformation is

$$C_6H_6 + 4.5H_2O \rightarrow 2.25CO_2 + 3.75CH_4. \quad (5.5)$$

Higher rates of degradation are reported under denitrifying conditions than under methanogenic conditions.[2] This is expected when one considers the thermodynamics of these reactions. The amount of energy obtainable from toluene with nitrate as an electron acceptor is 20 times higher than under methanogenic conditions.[8]

Oxygenated aromatic compounds such as alcohols, aldehydes, acids, and phenols are transformed by a reductive mechanism under anaerobic conditions.[2] Reduction occurs, converting the aromatic ring to an alicyclic ring, followed by hydrolytic cleavage and mineralization. The reduction can occur, in contaminated aquifers, under denitrifying, Fe(III) reducing, sulfate-reducing and methanogenic conditions.

5.3.1.3 Polynuclear Aromatic Hydrocarbons (PAHs)

Polynuclear aromatic hydrocarbons (PAHs) are compounds that have multiple rings in their molecular structure (Figure 5.4). They include the frequently found compounds such as naphthalene and anthracene and the more complex compounds such as pyrene and benzo(a)pyrene. Biodegradation of polynuclear aromatic hydrocarbons depends on the complexity of the chemical structure and the extent of enzymatic adaptation. In general, PAHs which contain two or three rings such as naphthalene, anthracene, and phenanthrene are degraded at reasonable rates when O_2 is present. Compounds with four rings such as chrysene, pyrene, and pentacyclic compounds, in contrast, are highly persistent and are considered recalcitrant. The factors which influence the degradation of PAHs under either aerobic or anaerobic conditions are (1) solubility of the PAH, (2) number of fused rings,

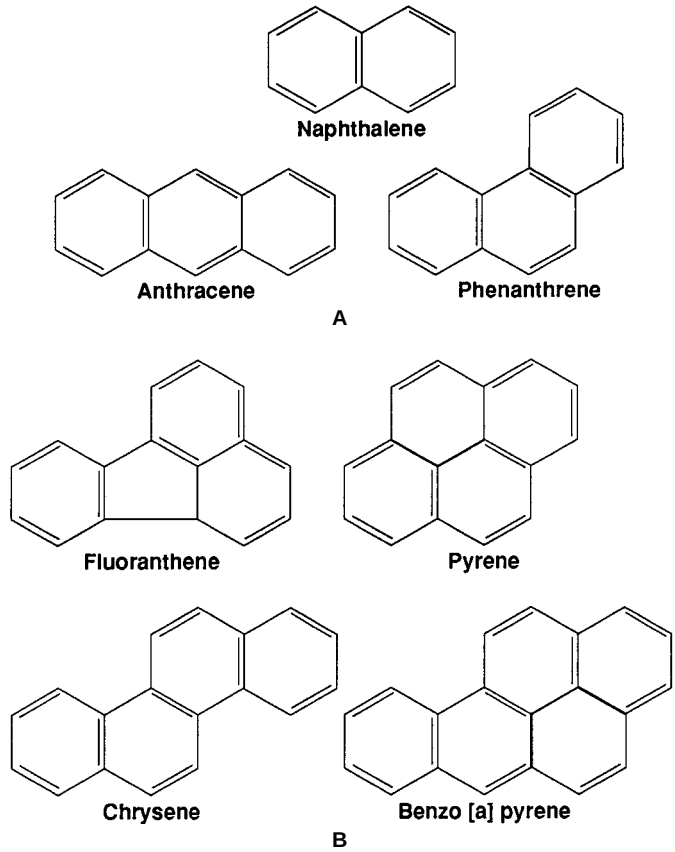


Figure 5.4 Structures of polynuclear aromatic hydrocarbons (PAHs). A. Mostly rapidly degraded PAHs. B. Slowly degraded or persistent PAHs.

(3) type of substitution, (4) number of substitution, (5) position of substitution, and (6) nature of atoms in heterocyclic compounds. The above factors are combined into a single parameter defined as structure–biodegradability relationship. Generalizations about structure–biodegradability relationships in aerobic environments do not seem to be applicable to anaerobic environments.[1]

Aerobic biodegradation of the two- and three-ring PAHs is accomplished by a number of soil bacteria. As the number of fused rings and the complexity of the substituted groups increase, the relative degree of degradation decreases. The influence of alkyl substituents is more difficult to predict.[2] One methyl addition significantly decreases the degree of degradation, and its effect varies with the substituted position. The addition of three methyl groups causes severe retardation of degradation.[9]

The importance of cometabolism for PAHs having four or more rings has been demonstrated by several investigations.[2] In fact, cometabolism may be the only metabolism mode for degradation of the heavier PAHs. Analog substrate enrichment may also be useful in enhancing the degradation of heavier PAHs. The presence of an analog substrate such as naphthalene will enhance the degradation of pyrene by many organisms.[2] Under this mode of metabolism the analog substrate is the primary substrate, and the suitable enzyme production becomes available to degrade the heavier PAH as the secondary substrate.

Many fungal species are known to degrade PAHs under aerobic conditions.[1,2] *Phanerochaete* and related fungi that have the ability to attack wood possess a powerful extracellular enzyme that acts on a broad array of PAHs. The enzyme is a peroxidase that, with H_2O_2 produced by the fungus, catalyzes a reaction that cleaves a surprising number of compounds.[1]

The fungus *Phanerochaete chrysosporium*, also known as white rot fungus, degrades many PAHs including benzo(a)pyrene, pyrene, fluorene, and phenanthrene.[2] Nitrogen-limiting conditions and lower pH (around 4.5) are favorable for this degradation.[10] The transformations by the fungus are slow, and the possibility of exploiting the catabolic activity under realistic field conditions have not been reported widely.

5.3.2 Chlorinated Organics Degradation

5.3.2.1 Chlorinated Aliphatic Hydrocarbons (CAHs)

Transformations of CAHs in the subsurface environment can occur both chemically (abiotic) and biologically (biotic). The major abiotic transformations include hydrolysis, substitution, dehydrohalogenation, coupling, and reduction reactions. Abiotic transformations generally result in only a partial transformation of a compound and may lead to the formation of an intermediate that is either more readily or less readily biodegraded by microorganisms.

Biotic transformation products are different under aerobic than anaerobic conditions. Microbial degradation of chlorinated aliphatic compounds can use one of several metabolism modes. These include oxidation of the compound for an energy source, cometabolism under aerobic conditions, and reductive dehalogenation under anaerobic conditions. However, with cometabolism, as with abiotic transformations, CAHs are generally transformed only partially by the microbial process.

With molecular oxygen as the electron acceptor, the one- to three-atom substituted chlorinated aliphatic compounds are transformed by three types of enzymes: oxygenases, dehalogenases, and hydrolytic dehalogenases.[11] With oxygenase the transformation products are alcohols, aldehydes, or epoxides. Dehalogenase transformation products are an aldehyde and glutathione. Hydrolytic dehalogenases will hydrolyze aliphatic compounds, yielding alcohols as a transformation product.

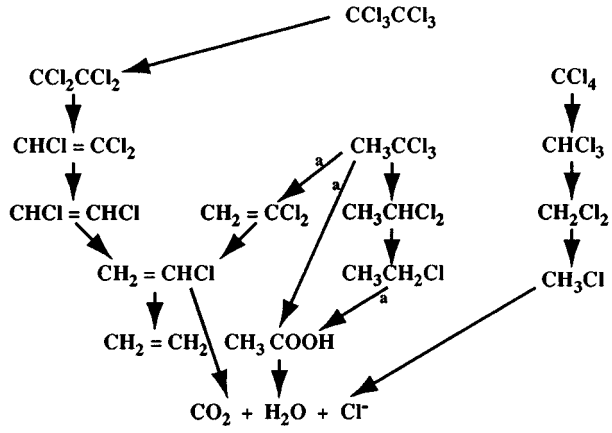
The higher chlorinated aliphatic compounds, where all available valences on carbon are substituted, such as tetrachloroethylene, have not been transformed under aerobic systems. The single-carbon saturated compound, dichloromethane, can be used as a primary substrate under both aerobic and anaerobic conditions, and completely mineralizes.[12] The two-carbon saturated CAH, 1,2-dichloroethane, can also be used as a primary energy source under aerobic conditions.[12] One unsaturated two-carbon CAH, vinyl chloride, has been shown to be available as a primary substrate for energy and growth under aerobic conditions.[12]

These observations indicate that only the less chlorinated one- and two-carbon compounds might be used as primary substrates for energy and growth, and that organisms that are capable of doing this are not widespread in the environment. The microbial transformation of most of the CAHs depends upon cometabolism.

5.3.2.1.1 Anaerobic Cometabolic Transformation of CAHs

Many chlorinated aliphatic compounds are transformed under anaerobic conditions. In the presence of a consortium of microorganisms, these compounds will be mineralized to CO_2, H_2O, and Cl^-. One of the predominant mechanisms for transformation of chlorinated aliphatic compounds is reductive dechlorination. The reductive process is usually through cometabolism. There are rare exceptions to the need for cometabolism, such as chloromethane serving as a primary substrate for a strictly anaerobic homoacetogenic bacterium.[2]

The pathways of anaerobic cometabolic, reductive dechlorination are shown in Figures 5.5 and 5.6. Figure 5.5 illustrates the various anaerobic biotic and abiotic pathways that chlorinated aliphatic compounds may undergo in the subsurface environment. Figure 5.6 also describes the anaerobic transformation of PCE and TCE under anaerobic conditions. During reductive dechlorination, the chlorinated compound serves as the electron acceptor.



(a). Abiotic transformation

Figure 5.5 Anaerobic transformations of chlorinated aliphatic hydrocarbons.

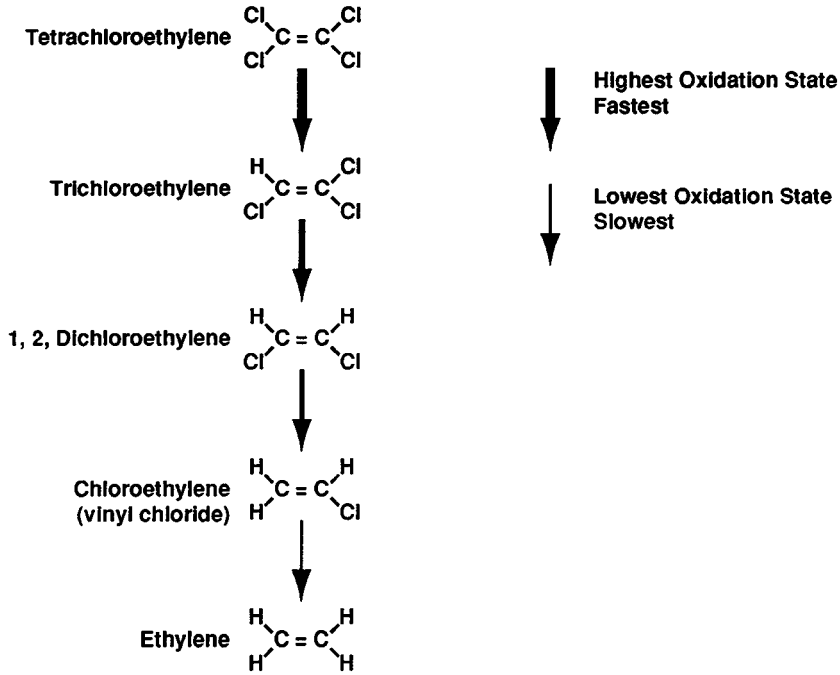


Figure 5.6 Anaerobic transformation of PCE and TCE.

The more chlorinated a compound is, the more oxidized the compound is, and the more susceptible it is to reduction (Figure 5.6). Reductive dehalogenation is carried out by electrons from the oxidation of the primary substrate.

Anaerobic transformations of tetrachloroethylene (PCE) and trichloroethylene (TCE) have been studied very intensely in the recent past.[1,2,12–14] General agreement exists that transformation of these two compounds under anaerobic conditions proceeds by sequential reductive dechlorination to dichloroethylene (DCE) and vinyl chloride (VC) (Figures 5.5 and 5.6); and in some instances, there is total dechlorination to ethene or ethane. Among the three

possible DCE isomers, 1,1-DCE is the least significant intermediate, and it has been reported that *cis*-1,2-DCE predominates over *trans*-1,2-DCE.

The pathways described in Figure 5.5 indicate that any chlorinated aliphatic compound can be transformed to innocuous end products under anaerobic conditions. However, the microbial transformations generally involve cometabolism such that other primary organic substrates and suitable microbial consortium must be present. Furthermore, as noted earlier, the rates of anaerobic transformations are much greater for the highly chlorinated compounds than for less chlorinated compounds; thus, the intermediates may persist longer in the environment. Also, some of the intermediates are more hazardous than the parent compounds, examples of which are the transformation of TCE to vinyl chloride and TCA to 1,1-DCE. Hence, with anaerobic transformation, all the right conditions must be present for complete transformation to innocuous end products to occur at sufficiently high rates.

The availability of other electron acceptors in anaerobic systems affects the reductive dechlorination process by competing with the chlorinated compounds for reducing potential. For example, sulfate and nitrate can inhibit the dechlorination, since microoganisms will tend to couple half reactions that yield the greatest free energy. Introduction of nitrate and sulfate was found to decrease the dechlorination rate of PCE under field conditions.[15,16] Reductive dechlorination rates were found to be the highest under highly reducing conditions associated with methanogenic reactions rather than under less reducing conditions associated with denitrifying conditions.[16] Degradation efficiencies under various anaerobic conditions for a few selected compounds are presented in Table 5.4.

Table 5.4 Degradation Efficiencies for Chorinated Compounds under Various Anaerobic Conditions

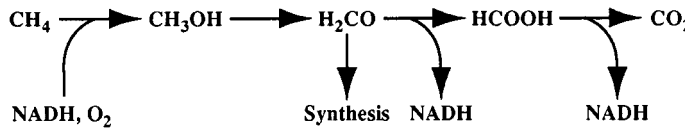
Compound	Removal efficiencies (percentage)		
	Denitrification	Sulfate Reduction	Methanogenic
PCE	0	13	86
Chloroform	0	0	95
1,1,1-TCA	30	72	>99
Carbon tetrachloride	>99	>99	>99

5.3.2.1.2 Aerobic Cometabolic Transformation of CAHs

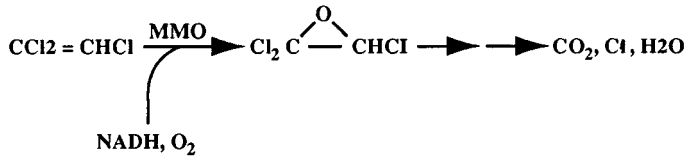
Until a few years ago, chlorinated compounds with two carbon atoms were considered nonbiodegradable under aerobic conditions. In the recent past, it was shown for the first time that TCE may be susceptible to aerobic degradation by methane utilizing bacterial communities.[17] The processes involved are illustrated by the pathways in Figure 5.7. Cometabolism of TCE is carried out by methanotrophic bacteria, which oxidize methane for energy and growth.

The responsible enzyme of methanotrophic bacteria, methane monooxygenase, catalyzes the incorporation of one oxygen atom from molecular oxygen into methane to produce methanol. The lack of substrate specificity of the monooxygenase enzyme results in its ability to oxidize a broad range of compounds, including chlorinated organic compounds. Methane monooxygenase fortuitously oxidizes TCE to form TCE epoxide, an unstable compound that chemically undergoes decomposition to yield a variety of products, including carbon monoxide, formic acid, glycoxylic acid, and a range of chlorinated acids.[12] Since these products cannot be further metabolized by methanotrophs, a community of microorganisms is necessary for mineralization to carbon dioxide, water, and chloride.

Although these oxygenase-generating microorganisms can oxidize chlorinated aliphatic compounds, engineering design of these systems are not simple. The cometabolite must always be present for sustained reactions. However, excessive methane and high oxygen concentrations will inhibit the oxidation of chlorinated compounds.[2] High methane, the



Methane Oxidation.



TCE Epoxidation.

Figure 5.7 Pathways of TCE cometabolism by methanotrophic microorganisms.

primary substrate, will hinder the reaction rate, since it will compete for the monooxygenase enzyme, making the enzyme unavailable for the target compounds. Furthermore, there is a potential for toxicity problems. It has been reported that trichloroethylene oxidation products are toxic to certain methanotrophs, and perchloroethylene (PCE) appears to inhibit trichloroethylene degradation.[2,11,18,19] Another serious limitation is that methanotrophs have not been reported to transform PCE or higher chlorinated aliphatic compounds, since the higher the degree of oxidation, the less easy it is to oxidize the compound.

Since the first report of TCE cometabolism,[17] many other groups of aerobic bacteria have been recognized as being capable of transforming TCE and other chlorinated aliphatic compounds. In addition to methane oxidizers, aerobic bacteria that are propane oxidizers, ethylene oxidizers, toluene oxidizers, phenol oxidizers, ammonia oxidizers, and vinyl chloride oxidizers also have been recognized to have the ability of cometabolizing CAHs.

Table 5.5 summarizes the discussion in the last few sections regarding the potential microbial transformations of chlorinated aliphatic hydrocarbon compounds.

Table 5.5 Potential for Biotransformation of Chlorinated Aliphatic Hydrocarbons as a Primary Substrate or through Cometabolism

Compound	Primary substrate		Cometabolism		Product
	Aerobic	Anaerobic	Aerobic	Anaerobic	
CCl_4			o	xxxx	$CHCl_3$
$CHCl_3$			x	xx	CH_2Cl_2
CH_2Cl_2	Yes	Yes	xxx		Mineralized
CH_3CCl_3			x	xxxx	CH_3CHCl_2
CH_3CHCl_2			x	xx	CH_3CH_2Cl
CH_2ClCH_2Cl	Yes		x	x	CH_3CH_2Cl
CH_3CH_2Cl	Yes		xx	a	
$CCl_2 = CCl_2$			o	xxx	$CHCl = CCl_2$
$CHCl = CCl_2$			xx	xxx	$CHCl = CHCl$
$CHCl = CHCl$			xxx	xx	$CH_2 = CHCl$
$CH_2 = CCl_2$			x	xx	$CH_2 = CHCl$
$CH_2 = CHCl$	Yes		xxxx	x	Mineralized

Note: o, very small, if any potential; x, some potential; xx, fair potential; xxx, good potential, xxxx, excellent potential; a, readily hydrolyzed abiotically.

From McCarty, P. L. and Semprini, L., Groundwater treatment for chlorinated solvents, in *Handbook of Bioremediation*, Norris, R. D., et al., Eds., Lewis Publishers, Boca Raton, FL, 1994. With permission.

5.3.2.2 Chlorinated Aromatic Hydrocarbons

Chlorinated aromatic hydrocarbons include a wide range of compounds present in the subsurface as contaminants and thus require remediation. These compounds are, to name a few, chlorophenols, chlorobenzenes, chloronitro benzenes, chloroaniline, polychorinated biphenyls (PCBs), and many pesticides.

Most chlorinated aromatic compounds that are degraded under aerobic conditions are probably acted upon through cometabolism.[2] It is also possible that a chlorinated aromatic compound is transformed to a toxic product that prevents further aerobic degradation. Complete aerobic mineralization of chlorinated aromatic compounds has been reported in the past.[2] However, the persistence of these compounds reflects the inability of many microorganisms to degrade these compounds. The nature and number of chlorine substitutions, and the substitution positions influence the extent of degradation. Degradation of chlorine-substituted aromatic compounds frequently does not follow the reaction pathways of the unsubstituted parent compounds.

Chlorinated aromatic hydrocarbons that are recalcitrant under aerobic conditions are sometimes degraded by one or more reductive dechlorinations under anaerobic conditions. Chlorinated organic compounds serve as the electron acceptors, and the primary substrate supplies the electron due to oxidation. Chlorine present at the *ortho*- and *para*-positions are more resistant to dechlorination than those at the *meta* position.[2] When the chlorine is removed, ring fission leads to methane and carbon dioxide.[20]

Methanogenic metabolism has successfully dechlorinated many aromatic organic compounds such as 3-chlorobenzoate, 2,4-dichlorophenol, and 4-chlorophenol.[2] Methanogenic cultures show preferential removal of *ortho*-chlorines, with *meta*- or *para*-chlorines removed at slower rates. Anaerobic dehalogenation and the final mineralization may require multiple species of microorganisms and reduction pathways. For example, 2,4-dichlorophenol was mineralized to CH_4 and CO_2 by as many as six species of microorganisms.[21]

Polychlorinated biphenyls (PCBs) are chlorinated aromatic compounds that are designated by numbers that represent the number of carbon atoms and the percentage of chlorine by weight. PCBs are also known under their trade name Aroclor™. For example, Aroclor 1252 contains 12 carbon atoms and has 52% chlorine by weight. PCBs are very insoluble in water and are mostly found only in soils and sediments.

No single organism is responsible for the degradation of multiple-chlorine PCBs. Both aerobic and anaerobic metabolism modes affect some biotransformation of PCBs. Analog substrate enrichments have produced varied results for PCB degradation.[2] Addition of biphenyl as an analog substrate had significant effect on the degradation of Aroclor 1242.[22] Analog enrichment, however, did not yield positive results in studies with Aroclor 1254.[23]

As noted earlier, anaerobic metabolic modes have a significant advantage over aerobic modes for PCBs. Dechlorination of the highly chlorinated Aroclor 1260 even occurs to a significant extent under anaerobic conditions.[2]

Fungi known to degrade wood, such as white rot fungi, have been documented to mineralize tri-, tetra-, and pentachlorophenol (PCP).[24] It was also reported that a consortium of microorganisms present in soil can completely mineralize PCP.[2]

5.4 BIODEGRADATION KINETICS AND RATES

Biodegradation of organic compounds, their pathways and the kinetics of defined enzymatic degradation steps, has generally been determined in well-defined, optimal laboratory conditions such as aqueous systems or shake flask experiments using water–soil/sediment suspensions or batch experiments. Mainly, these data have been used for model approaches, but they are hardly relevant for biodegradation rates *in situ*. Half-life periods as a parameter

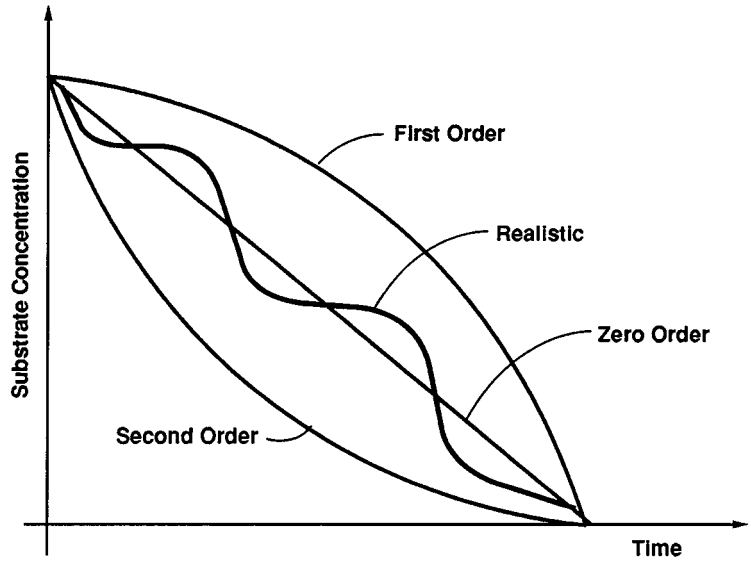


Figure 5.8 Microbial degradation kinetics order.

and first-order kinetics as a function are most commonly used for describing degradation of contaminants in the subsurface. Degradation, however, strongly depends on the site-specific environmental conditions under consideration. Measuring half-life is rather easy, since it is based on disappearance, but it does not take into account the difference between one transformation step and complete mineralization.

Kinetic models for microbial degradation are based on substrate concentration and biomass.[25] This leads to three types of kinetic order for biodegradation in natural environments, often based on empirical knowledge, and thus reflecting the rudimentary level of knowledge about microbial populations and their activity in these environments. When the substrate is completely available (i.e., its availability is not rate-limiting), the degradation only depends on the activity of the microorganisms following logarithmic growth. The degradation follows zero-order kinetics: logarithmic disappearance. A process follows first-order kinetics when the rate of biodegradation of a compound is directly proportional to its concentration. The second-order approach, in which the first-order kinetics is related to the population density, is the most realistic one. Lack of detailed stepwise degradation information may be one of the reasons why the occurrence of nonlinear reactions is presumed. This phenomena is described by equation (5.6)[1] and Figure 5.8.

$$\frac{-dC}{dt} = kC^n \tag{5.6}$$

where C = substrate concentration
t = time
k = rate constant for chemical disappearance
n = a fitting parameter.

The response of the microbial community toward organic compounds does not depend on total concentration, but mainly on the water soluble concentration. Bioavailability of a compound is of extreme importance, because it frequently accounts for the persistence of compounds that are biodegradable and that might otherwise be assumed to be readily degraded.

The unavailability of a compound could result from its sorption to solids in the environment, its presence as nonaqueous phase liquid (NAPL), its entrapment within the physical matrix of the soil, and diffusional limitations.

When two or more different sequential microbial populations are required for complete degradation, longer than normal acclimatization times may be involved. For difficult to degrade compounds, this is rather a rule than an exception. Different kinetics of the various degradation steps and this acclimatization time are the reasons why the overall disappearance seems to follow a cyclic pattern (Figure 5.8). It can be assumed that nonlinear responses in reality are rather a rule than exception. Other factors that may impact the bioavailability, and thus the kinetics, of biodegradation are weathering, sequestering, and complexation of substrate.[1] *Weathering* of a contaminant results in easily biodegradable compounds being degraded early and formation of an aged residue. *Sequestering* of a compound occurs when a compound becomes less available or essentially wholly unavailable for biodegradation when it enters or is deposited in a micropore that is inaccessible to microorganisms. *Complexation of a compound* affects biodegradation when the contaminant forms insoluble complexes in association with inorganic or organic substances present in the environment.

Another factor that influences the kinetics of biodegradation is the chemical structure of the contaminant of concern. Most naturally formed compounds are biodegradable, because the relatively few catabolic pathways of microorganisms would have been exposed to these natural compounds. A synthetic chemical that is not a product of biosynthesis will be degraded only if an enzyme or an enzyme system is able to catalyze the conversion of this compound to an intermediate or a substrate which is able to participate in existing metabolic pathways. The greater the difference in structure of the xenobiotic form from the compounds produced in nature, the less is the likelihood for significant biodegradation.

Various approaches have been used to predict biodegradability in the past. These approaches include empirical, theoretical, and experimental methods.[1] The experimental methods include laboratory bench-scale experiments based on the disappearance of the compound; respirometric studies based on the oxygen uptake of aerobic microorganisms either in the laboratory or in the field; and measurement of half-lives based on the degradation of compounds.

Empirical approaches include predicting biodegradability from the properties of a compound similar to a substrate in known metabolic pathways, and predicting biodegradability based on the chemical and physical properties of a compound, such as water solubility, boiling point, melting point, molecular weight, density, partition coefficient, etc. It should be noted that empirical biodegradability predictions are qualitative at most.

Theoretical predictions of biodegradability are based on molecular topology,[1] which deals with structural features of contaminant molecules such as shape, size, presence of branching, and types of atom-to-atom connections. Of particular interest in molecular topology is molecular connectivity, which can be determined from the structural formula of the compound.

5.5 ENVIRONMENTAL FACTORS

Microbial populations capable of degrading contaminants in the subsurface are subjected to a variety of physical, chemical, and biological factors that influence their growth, their metabolic activity, and their very existence. The properties and characteristics of the environments in which the microorganisms function have a profound impact on the microbial population, the rate of microbial transformations, the pathways of products of biodegradation, and the persistence of contaminants. The impact of site-specific factors is evident from studies showing that a specific compound is biodegraded in samples from one but not another environment.[1]

A vast amount of information exists on the biochemical activities of microorganisms grown in pure or mixed cultures at various concentrations in laboratory media. This research has created a foundation for the understanding of the nutrition, population dynamics, and metabolic potential of microorganisms under controlled laboratory conditions. However, in nature, microorganisms are exposed to enormously different conditions. They may have an insufficient supply of inorganic nutrients; a paucity of essential growth factors, temperatures, and pH values at their extremes of tolerance; and contaminant levels that stress the microbial population. Contaminants at very high levels can retard the growth, inhibit the metabolic activity, and may also result in loss of viability. As a consequence, extrapolations from laboratory tests, performed under controlled conditions, to field conditions may be fraught with peril.

5.5.1 Microbial Factors

The microbial population of the soil is made up of five major groups: bacteria, actinomycetes, fungi, algae, and protozoa. Bacteria are the most abundant group, usually more numerous than the other four combined. Although transformations similar to those of the bacteria are carried out by the other groups, the bacteria stand out because of their capacity for rapid growth and degradation of a variety of contaminants. Classification of bacteria has been proposed in various forms to meet different objectives: (1) ability to grow in the presence or absence of oxygen, (2) cell morphological structure, and (3) type of energy and carbon sources.

The ability to grow in the presence or absence of oxygen is an important biochemical trait which has led to three separate and distinct categories: *aerobes*, which must have access to O_2; *anaerobes*, which grow only in the absence of O_2; and *facultative anaerobes*, which can grow either in the absence or presence of O_2.

Three morphological types are known, the *bacilli* or rod-shaped bacteria, which are the most numerous, the *cocci* or spherical-shaped cells, and the *spirilla* or spirals. The latter are not common in soils. Some of the bacilli persist in unfavorable conditions by the formation of endospores that function as part of the normal life cycle of the bacterium. These endospores often endure in adverse environments because of their great resistance to both prolonged desiccation and to high temperatures. Spore-forming genera are present among the aerobic and anaerobic bacteria. The endospore can persist in a dormant state long after the lack of substrate or water has led to the death of vegetative cells. When conditions conducive to vegetative growth return, the spore germinates and a new organism emerges.

Microorganisms are divided into two broad classes with respect to their energy and carbon sources. *Heterotrophic* forms, which require organic substrates to serve as sources of energy and carbon, dominate the soil microflora. *Autotrophic* microorganisms obtain their energy from sunlight or by the oxidation of inorganic compounds and their carbon by the assimilation of CO_2. Autotrophs are of two general types: *photoautotrophs* whose energy is derived from sunlight, and *chemoautotrophs* which obtain the energy needed for growth from the oxidation of inorganic materials.

There is frequently an initial period, also known as the acclimatization lag, during biodegradation of contaminants. During this period, no obvious biotic changes of contaminant levels take place. This period may be due to various reasons, and the causes may be in the indigenous microbial communities. The starting biomass may be so low that no appreciable degradation can happen until a critical biomass concentration is reached or the total microbial population may be abundant, but the specific degrading populations may need to be enriched. On other occasions, the contaminant must induce requisite enzyme or a new enzyme needs to be synthesized. Sometimes, the reasons for the initial lag period may lie in the contaminants themselves. The contaminants may be present in such low concentrations that they will not induce the relevant enzymes, or their chemical structure may be so unusual that they cannot

interact with the active enzyme sites. The lag for the degradation of a specific contaminant can also occur due to the preferential depletion of other substrates first.

Measurement of the indigenous microbial activity is one method for evaluating potential toxic or inhibitory conditions at a site. Low bacteria counts can indicate a potential toxicity problem or a stressed microbial population. Groundwater bacterial counts range from 10^2 to 10^5 colony forming units (CFU) per milliliter of sample. Typical soil microbial counts range from 10^3 to 10^7 CFUs per gram of soil. Higher counts indicate a healthy microbial population. Counts below 10^3 organisms per gram of soil at contaminated sites may indicate a stressed microbial population.

5.5.2 Nutrients

Carbon makes up a large fraction of the total protoplasmic material of a microbial cell. Carbohydrates, proteins, amino acids, vitamins, nucleic acids, purines, pyrimidines, and other substances constitute the cell material. In addition to carbon, cell material is mainly composed of the elements hydrogen, oxygen, and nitrogen. These four chemical elements constitute about 95% by weight of living cells. Two other elements, phosphorus and calcium, contribute 70% of the remainder. The elemental composition of microbial cells on a dry weight basis is presented in Table 5.6.

Table 5.6 Elemental Composition of Microbial Cell on a Dry Weight Basis

Element	Percentage of dry weight
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All others	0.3

The microbial requirements for nutrients are approximately the same as the composition of their cells (Table 5.6). The chemical structure of bacteria is often expressed as $C_5H_7O_2N$ with only minor, but important, traces of other atoms. Carbon is usually supplied by organic substrates—organic contaminants in the case of bioremediation—for the heterotrophic microorganisms. Autotrophic microorganisms obtain their carbon supply from inorganic sources such as carbonates and bicarbonates. Hydrogen and oxygen are supplied by water. Usually, the nutrients in short supply are nitrogen, phosphorus, or both. Nearly always, the supply of potassium, sulfur, magnesium, calcium, iron, and micronutrient elements is greater than the demand. These micronutrients are present in most soil and aquifer systems.

It is widely believed that only one nutrient element is limiting at any given time, and that only when that one deficiency is overcome does another nutrient become limiting. This condition is stated by Liebig's law of the minimum: The essential constituent that is present in the smallest quantity relative to the nutritional requirement of microorganisms will become the limiting factor of growth. This law can be expanded to include the electron acceptor also.

Even in the absence of added N and P, biodegradation will continue in the subsurface, albeit at a slow rate. This phenomenon is due to the recycling of the elements as they are assimilated into microbial cells and then are converted back to the inorganic forms due to

the death and lysis of microbial cells. Under such circumstances, the rate of biodegradation will be limited and will be impacted by the rate at which the limiting nutrient is recycled.

Many microorganisms also require some substances that are part of the cell structural building blocks, at trace quantities. These substances, known as the growth factors, are organic molecules such as amino acids, vitamins, or other structural units. Growth factors are not essential nutrients, but they stimulate the species of organisms that need them.

5.5.3 Physical–Chemical Factors

The activities of microorganisms are markedly affected by their physical–chemical environment. Environmental parameters such as temperature, pH, moisture content, and redox potential will determine the efficiency and extent of biodegradation.

5.5.3.1 Temperature

As temperature increases, the rates of chemical as well as biochemical reactions generally increase. This phenomenon is referred to as Arrhenius behavior (Figure 5.9A). The same phenomenon also occurs with microorganisms and the myriad of chemical and biochemical reactions that constitute "microbial activity," but only to a point. While the rates of abiotic chemical reactions might increase in an unbounded fashion with increasing temperature, this is not the case with microbial activity. Beyond some optimum temperature, the activity of any organism declines precipitously. At the lower end of the temperature range, most bacteria stop metabolic activities at temperatures just above the freezing point of water.

The decline of microbial activity at temperatures beyond the optimum is usually explained in terms of the three-dimensional shapes of enzymes and the effects of temperature on membrane integrity. Three categories of microorganisms are defined, based upon temperature optima (Figure 5.9B):

- *Psychrophiles:* Psychrophilic (or cryophilic) organisms have an optimum temperature of 15 ± 5°C, and a minimum temperature of 0°C or below. Strict psychrophiles usually die if exposed even temporarily to room temperatures. On the other hand, there are organisms with optima at 25 to 30°C, but which can grow at 0°C; these are sometimes called *facultative psychrophiles*. Psychrophiles usually possess membranes rich in unsaturated fatty acids, a feature which is alleged to provide a more fluid structure at low temperatures.
- *Mesophiles:* Mesophilic organisms have an optimum temperature between 25°C and 40°C. Most of the microorganisms that inhabit the subsurface are mesophiles. Microorganisms commonly found effective in bioremediation perform over a temperature range of 10 to 40°C. For many regions of the country, groundwater temperatures remain reasonably constant throughout the year at around the mean air temperature for the region.[27]
- *Thermophiles:* Thermophilic organisms have temperature optima above 45°C. For example, there are thermophilic methanogens that prefer temperatures of 55 to 60°C. Some are facultative thermophiles, in that their range extends into the mesophilic zone. Thermophiles have membranes rich in saturated fatty acids. The soil surface temperature around noontime during a summer day could reach 50 to 70°C.

5.5.3.2 pH

The pH affects the microorganism's ability to conduct cellular functions, cell membrane transport, and the equilibrium of catalyzed reactions by having an impact on the three-

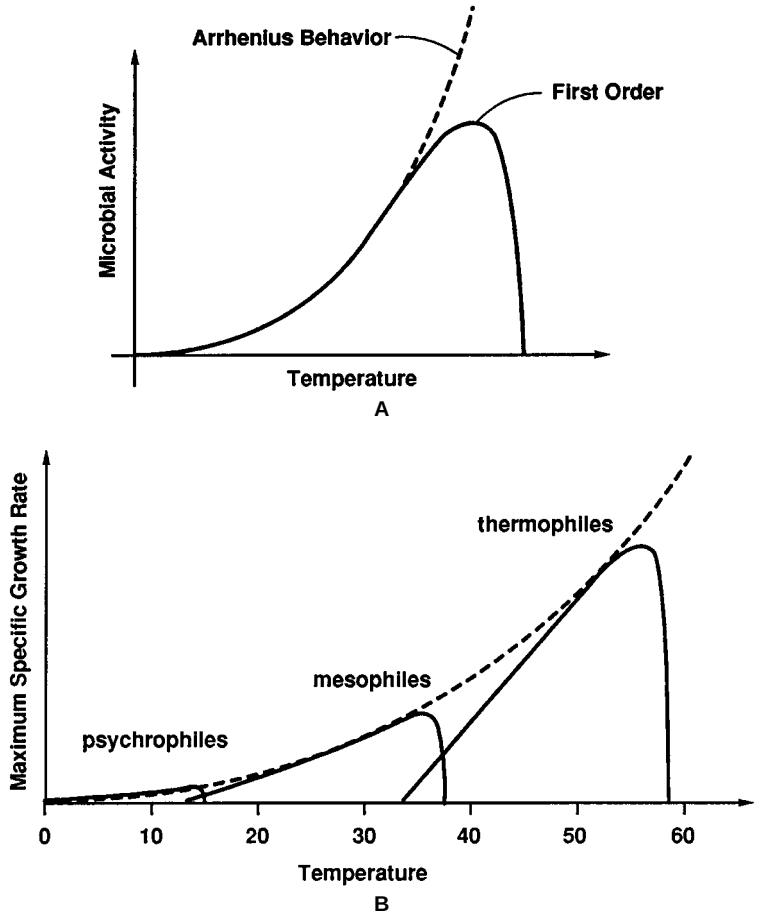


Figure 5.9 A. Microbial activity with temperature. B. Temperature dependency of growth rate of various microorganisms.

dimensional conformation of enzymes and transport proteins of microbial cells. It also affects the protonmotive forces responsible for energy production within the cell.

Most natural environments possess pH values in the range between 5 and 9. Therefore, it is not surprising to find that most microorganisms have evolved with pH tolerances within this range. Most bacteria tolerate pH 5 to 9 but prefer pH 6.5 to 7.5. There are *acidophilic* bacteria such as *Thiobacillus thioxidans*, which have pH optimum near 2.5. Also, there are *alkalophilic* bacteria that can function at pH 10 to 12.

Metabolic activities of microorganisms produce organic acids and HCl from the degradation of organic compounds (chlorinated organics). When high concentrations of organic compounds are present in the subsurface with low alkalinity, pH control may be necessary to sustain continued biodegradation.

5.5.3.3 Moisture Content

Moisture is a very important variable relative to bioremediation. Moisture content of soil affects the bioavailability of contaminants, the transfer of gases, the effective toxicity level of contaminants, the movement and growth stage of microorganisms, and species distribution.[2]

Soil moisture is frequently measured as a gravimetric percentage or reported as field capacity. Evaluating moisture by these methods provides little information on the "water

availability" for microbial metabolism. *Water availability* is defined by biologists in terms of a parameter called water activity(a_w):

$$a_w = \frac{RH}{100} = \frac{P_w}{P_w^o} \tag{5.7}$$

where RH = relative humidity of a covered system
P_w^o = vapor pressure of pure water at the temperature of the system
P_w = vapor pressure at equilibrium with water in the system.

In simple terms, the water activity is the ratio of the system's vapor pressure to that of pure water (at the same temperature). Pure water has a water activity of 1.0, seawater 0.98, and dried fruit 0.7.

Microbial transport of water through the bacterial plasma membrane is a passive process, governed strictly by diffusion and the gradient in a_w across the membrane.

5.5.3.4 Oxidation-Reduction (Redox) Potential

The redox potential is a measure of how oxidizing or reducing an environment is. Redox potential is sometimes denoted by the symbol E_H. E_H greater than zero is commonly interpreted to be an oxidizing environment, and E_H less than zero, a reducing environment. The practical range of E_H in the natural environment is from +800 mV (high O_2, with no O_2-depleting processes) to about –400 mV (systems high in H_2). Redox potential is measured by use of a platinum electrode, in conjunction with some reference electrode. Unfortunately, interpretation of measured E_H values is very difficult, because natural systems are seldom at equilibrium.

Table 5.3 presents the impact of redox potential on various mechanisms of microbial transformation of contaminants during bioremediation. However, it should be noted that the concentrations of particular oxidants or reductants will affect microbial metabolic activity regardless of the redox potential. The concentration levels of oxidants or reductants will influence the enzymatic activity via effects on three-dimensional conformation.

5.6 *IN SITU* BIOREMEDIATION SYSTEMS

The most significant challenge in *in situ* bioremediation is introducing into the subsurface environment the reagents needed by microorganisms and mixing them with the contaminants to be degraded. Much of the methodology usually associated with *in situ* bioremediation can be attributed to the pioneering research and development carried out by Richard L. Raymond and Sun Tech in the 1970s. By the mid-1980s, the potential of *in situ* bioremediation was widely accepted in the remediation industry. In the last few years, there has been an explosion of activity in bioremediation which now incorporates a wide range of processes in the *in situ* environment.

A continuing source of debate among practitioners of bioremediation are the concepts of *biostimulation* and *bioaugmentation*. Biostimulation consists of adding nutrients, such as nitrogen and phosphorus, as well as oxygen and other electron acceptors, to the microbial environment to stimulate the activity of microorganisms. Bioaugmentation involves adding exogenous microbes to the subsurface where organisms able to degrade a specific contaminant are deficient. Microbes may be "seeded" from populations already present at a site and grown in an above-ground reactor, or specially cultivated strains having known capabilities for degrading a specific contaminant.

Most bioremediation systems employ some form of biostimulation. However, there is a significant resistance in the industry to use bioaugmentation. This resistance stems from the *ubiquity principle*, which states that all microorganisms are ever-present in the subsurface environment. Another argument against bioaugmentation is that indigenous organisms already present at the contaminated site would have developed the enzyme systems to degrade the target contaminants. Furthermore, the limitation of distributing the exogenous microbial cultures in the subsurface and the question of long-term survivability of these lab-grown cultures under field conditions also discourage bioaugmentation. Bioaugmentation may play a prominent role in bioremediation when the release of genetically engineered organisms is permitted.

5.6.1 Screening Criteria

Prior to designing an *in situ* bioremediation system, the feasibility of biodegradation should be carefully evaluated. This evaluation should include the ease or difficulty of degrading the target contaminants, the ability to achieve total mineralization, and the environmental conditions necessary to implement the process. There are various factors that should be incorporated into this evaluation process.

- *Biodegradability of contaminants:* Years of experience and research has established the degradation pathways of many specific contaminants. Contaminant characteristics and structure also will provide answers in terms of biodegradability. As an illustration, the following compounds have been listed with the compounds easiest to degrade at the top and the difficult ones at the bottom.

Simple hydrocarbons, C1–C15	very easy
Alcohols, phenols, amines	very easy
Acids, esters, amides	very easy
Hydrocarbons, C12–C20	moderately easy
Ethers, monochlorinated hydrocarbons	moderately easy
Hydrocarbons, greater than C20	moderately difficult
Multichlorinated hydrocarbons	moderately difficult
PAHs, PCBs, pesticides	very difficult

- *Mineralization potential of the compounds:* A review of pertinent reaction pathways will provide insight as to whether the contaminant will be utilized as a primary substrate or whether cometabolic reactions are necessary.
- *Specific microbial, substrate, and other conditions:* Of prime importance is the availability of carbon and energy in the contaminated environment. Electron acceptor availability and the redox condition should be carefully determined. In addition, the presence of microorganisms capable of degrading the contaminants, in sufficient numbers, should be evaluated. Total plate counts, specific degraders counts, and laboratory and *in situ* respiration tests can be utilized to perform this evaluation.
- *Availability of nutrients:* In general, the concentration levels of only N and P are determined.
- *Site's hydrogeologic characteristics:* Hydraulic conductivity, thickness of the saturated zone, homogeneity, and depth to the water table are parameters that should be factored into the design of the system. Distribution and transport of added nutrients and electron acceptors will be heavily influenced by the site hydrogeology.
- *Extent and distribution of contaminants:* This assessment with the site hydrogeologic parameters are the key components for developing the engineering design of the "subsurface bioreactor."

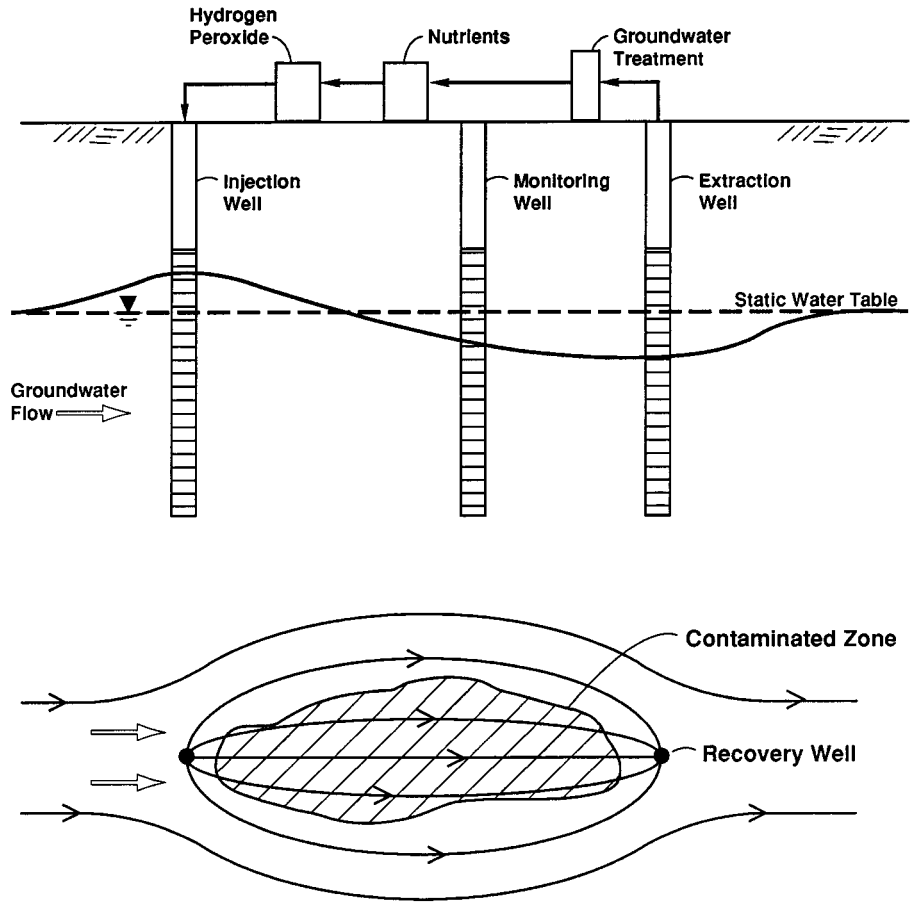


Figure 5.10 Description of the Raymond process.

- *Biogeochemical parameters:* Measurements of various biogeochemical parameters such as dissolved oxygen (DO), redox potential, CO_2, and other parameters such as NH_4^+, NO_3^-, SO_4^{2-}, S^{2-}, and Fe^{2+} will give an indication of the existing (natural or intrinsic) microbial metabolic activity at the site.

5.6.2 Raymond Process

The Raymond process shown in Figure 5.10 includes groundwater recovery wells, aboveground treatment, amendment with nutrients and possibly an electron acceptor, and reinjection of the amended groundwater.[28] This concept was developed on the premise that for most *in situ* bioremediation systems, the rate-limiting step is the rate of introduction of the electron acceptor. In the process shown in Figure 5.10, hydrogen peroxide was often used as the means of introducing oxygen to enhance the rate of aerobic biodegradation.

The perceived advantage of hydrogen peroxide is that due to its high levels of solubility compared to dissolved oxygen, a significant amount of available oxygen could be introduced into the aquifer. It was also believed that due to the high solubility, hydrogen peroxide could travel a long distance from the point of injection before being consumed. However, it was found that due to the instability of hydrogen peroxide in the presence of Fe and colloids, most of the H_2O_2 decomposed within a short distance from the point of injection.

In the Raymond process, the saturated zone of the contaminated area was manipulated to affect a "closed loop" flow system with a significant increase in groundwater flow rates.

In this manner, added O_2 and nutrients were transported faster than the natural groundwater flow velocities. Microbial populations and rate of degradation can be increased by several orders of magnitude within this "subsurface bioreactor." This configuration will also provide hydraulic containment of the plume at the downgradient edge.

One significant disadvantage of this process is the inefficient utilization of the injected H_2O_2. Less than 10 to 20% of the injected H_2O_2 only will be consumed by the microorganisms for biodegradation. The rest is lost due to the escape of the O_2 produced into the soil gas above the water table.

Injection of air into the saturated zone for the purpose of introducing O_2 to enhance biodegradation (biosparging) is described in Chapter 4.

5.6.3 Denitrification-Based *In Situ* Bioremediation

One promising alternative to the saturation limitations or high costs of the major alternative forms of oxygen involves the use of nitrate as the oxygen acceptor. In this process, the biodegradative activities of denitrifying organisms are enhanced, resulting in biodegradation of the target organic contaminants along with the transformation of NO_3^- to N_2. Nitrate feedstocks can thus be substituted for oxygen feedstocks in the groundwater manipulation system described in the previous section.

During *in situ* biosparging, the consumption of O_2 is relatively fast and the rate of O_2 transfer from the injected air to the aqueous phase is slow, due to low solubility of O_2 in water. Expansion of the aerobic zone is limited by the rate of O_2 supply to the aqueous phase. Anaerobic conditions are expected to persist within aerobically treated aquifers, especially in relatively impermeable zones and zones further away from the injection wells. The overall degradation efficiency can be increased by using nitrate, which is much more water-soluble than O_2 (9200 mg/l as $NaNO_3$ vs. 8 to 10 mg/l as O_2). The reducing equivalents that can be introduced into an aquifer using saturated sodium nitrate solution is approximately 50 times higher than with a saturated oxygen solution. However, due to regulatory and microbial toxicity considerations, the nitrate feedstock solution concentration should be significantly lower than saturated concentration.

Design of the *in situ* bioremediation system can be accomplished without downgradient groundwater extraction and upgradient injection. However, multiple injection points may be required to enhance the distribution and transport of the added reagents. Infiltration galleries can be also used to introduce the NO_3 and nutrients solution into the contaminated plume. Infiltration alone may limit the availability of the added reagents in the deeper zones of the contaminated plume.

Possible injection scenarios are shown in Figures 5.11 and 5.12. Based on Figure 5.11, if only injection wells are used, distribution and transport of reagents will be less effective than using both injection and extraction wells. When injection and extraction wells are used, lateral and vertical dispersion of the injected reagents will be increased, and thus the effectiveness of the "subsurface" bioreactor will be enhanced.

Denitrification-based *in situ* bioremediation has been field tested, and limited information is available in the literature. In one field study, a gasoline-contaminated plume was bioremediated by the injection of nitrate-spiked water.[29] In another study, nitrate addition into treatment cells within a JP-4 jet fuel contaminated plume resulted in degradation of specific contaminants.[30]

5.6.4 Pure Oxygen Injection

Providing an electron acceptor such as oxygen for enhanced bioremediation often becomes the critical limiting factor during system design. Continuous or intermittent oxygen delivery into the saturated zone is a challenging task, with field options primarily limited to

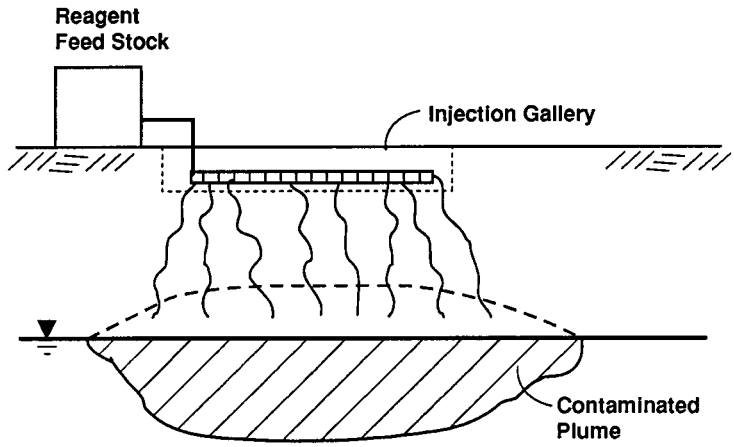


Figure 5.11 Injection of reagents via injection gallery.

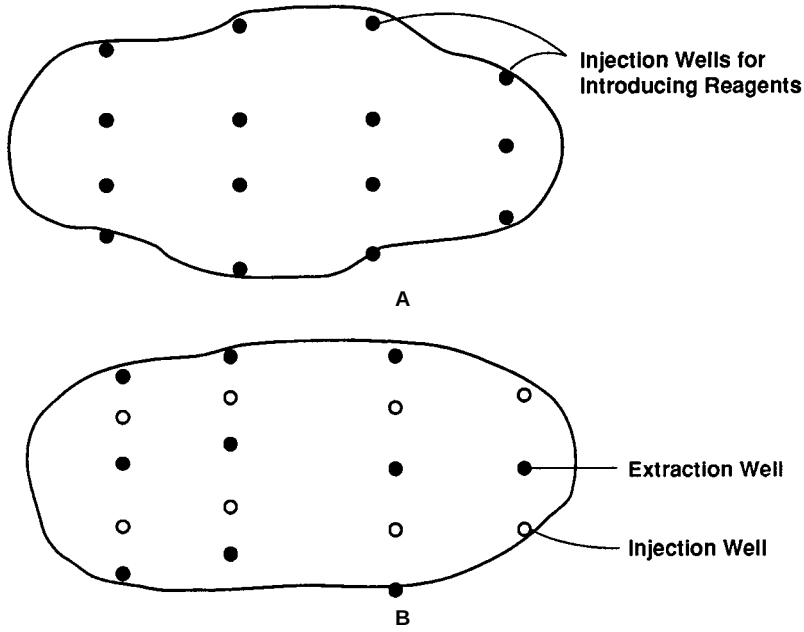


Figure 5.12 Injection well configurations for introducing reagents for bioremediation. A. Injection wells alone. B. Injection and extraction wells.

sparging air and adding hydrogen peroxide. These two options have been discussed in detail in Chapters 4 and 8, respectively.

An innovative technique to inject pure oxygen in the form of microbubbles has been reported in the literature.[31] A coarse soil matrix in the saturated zone is preferred for this technique to provide both a high permeability for flowing groundwater and a suitable saturated matrix for adhering and retaining microbubbles. The use of oxygen microbubbles for *in situ* bioremediation has the advantages of increased oxygen transfer rate to flowing groundwater (DO pickup), and increased oxygen utilization (percent of O_2 injected) compared to air injection.

The microbubbles are typically made from a low-surface-tension water containing 100 ppm or more of an appropriate surfactant. The microbubbles upon generation resemble a thick cream with much of the volume made up of microbubbles. The bubbles are generated by a colloidal gas apron.

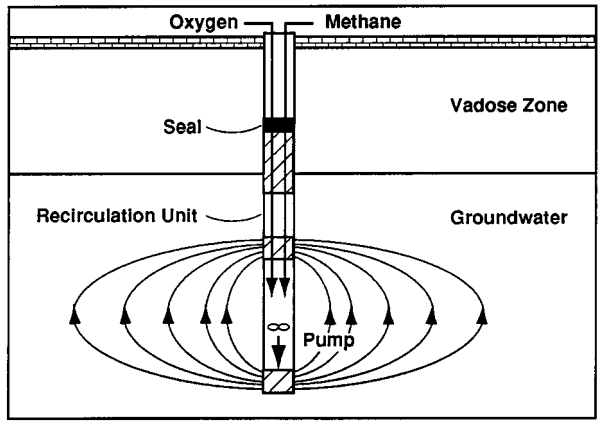


Figure 5.13 Subsurface recirculation system for methane and O_2 injection. (From McCarty, P. L. and Semprini, L., Groundwater treatment for chlorinated solvents, in *Handbook of Bioremediation*, Norris, R. D., et al., Eds., Lewis Publishers, Boca Raton, FL, 1994. With permission.)

In a field study, it was demonstrated that approximately 15 to 20% of the oxygen injected can be dissolved into the flowing groundwater.[31] With 10% committed to biodegrading the surfactant, a minimum of 5 to 10% net utilization was available for biodegrading contaminated groundwater.

5.6.5 Methanotrophic Biodegradation

Injection of methane and other required nutrients can enhance the cometabolic degradation of TCE and some other chlorinated aliphatic hydrocarbons. The methane provides the necessary material substrate for the indigenous microorganisms to produce the enzyme methane monooxygenase which, in turn, will degrade the TCE.

Typical injection rates of methane lie in the range of 1 to 4% in the methane–air mixture. Since methane is injected as a gaseous reagent, it is prudent to select the nutrients also in the form of gases. Nitrogen in the form of NH_3 gas or nitrous oxide and phosphorus in the form of triethyl phosphate can be used as nutrient sources.

In a field demonstration test using a horizontal injection well, 300 ft long and 35 ft below the water table, it was determined that 40% of the contaminant removal was achieved through methanotrophic cometabolic biodegradation.[32] The rest was removed by volatilization as a result of air injection.

In another field demonstration study of methanotrophic degradation of CAHs, the stimulation of indigenous methanotrophs was accomplished through methane and oxygen addition.[12] In this case methane and oxygen were added to the extracted groundwater and injected in the dissolved form. Concentrations of methane and oxygen were in the range of 16 to 20 mg/l and 33 to 38 mg/l, respectively. The conceptual application of this process can be implemented the same way as that shown in Figure 5.10. Another possible system for delivering the needed chemicals is subsurface groundwater circulation (Figure 5.13). This eliminates the need to pump contaminated groundwater to the surface treatment and reinjection. Methane and oxygen would be introduced directly into the well, which has a pump to induce flow from the bottom of the well and release at the top screen intersecting the water table. Instead of a pump, air injection to effect air lifting of the water will serve the dual purpose of pumping the water and introducing O_2. Multiple recirculation wells installed in a line across the direction of the groundwater flow will serve as a biologically reactive zone.

The study showed that the rates and extents of transformation were compound-specific and also that the cometabolic transformation was strongly tied to methane utilization; upon stopping methane addition, transformation rapidly ceased.[12] The percentage of transforma-

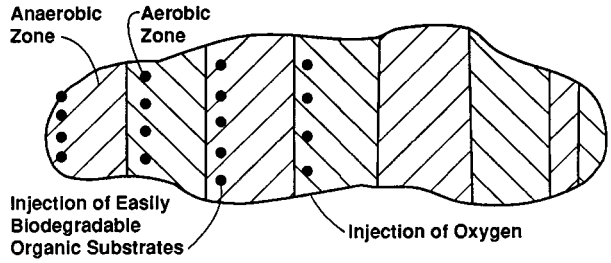


Figure 5.14 Anaerobic–aerobic sequential biodegradation.

tions achieved were TCE, 20%; *cis*-1,2-DCE, 50%; *trans*-1,2-DCE, 90%; and vinyl chloride, 95%. The difference in rates for *cis*-DCE and *trans*-DCE shows that a small change in chemical structure can have a large effect on the cometabolic transformation rate.

At the same site, higher rates of TCE degradation were accomplished by aerobic cometabolic stimulation of phenol-utilizers through phenol and oxygen addition.[12] The cometabolic transformation was strongly tied to the amount of phenol utilized and transformations achieved were TCE, 85%; and *cis*-DCE, over 90%.

5.6.6 Enhanced Anaerobic Biodegradation

Addition of easily biodegradable organic substrates will enhance the reductive dechlorination of many of the chlorinated aliphatic hydrocarbons. Many organic substrates such as acetate, butyric acid, lactic acid, methanol, ethanol, vitamin B_{12}, and sucrose have been shown to be effective in acting as the primary substrate to enhance the anaerobic cometabolic transformations. However, anaerobic dechlorination reaction rates are slower compared to the possible aerobic transformations of some of the intermediates. Hence, an anaerobic–aerobic sequential transformation will be able to achieve mineralization at a much faster rate than completely anaerobic pathways. If the contamination plume to be remediated is large, multiple anaerobic–aerobic sequencing segments can be implemented to achieve faster cleanup times (Figure 5.14).

5.6.7 Oxygen Release Compounds

Formulations of very fine, insoluble magnesium peroxide (MgO_2) release oxygen at a slow, controlled rate when hydrated. Their use has been demonstrated to increase the dissolved oxygen concentrations within contaminated plumes, and thus enhance the rate of aerobic biodegradation.[33,34] Magnesium peroxide releases oxygen when it comes in contact with water as shown by the following equation:

$$MgO_2 + H_2O \rightarrow \tfrac{1}{2} O_2 + Mg(OH)_2 . \qquad (5.8)$$

The byproducts of the reaction are oxygen and magnesium hydroxide, which will also help in maintaining moderate pH levels within the contaminated plume.

MgO_2 is normally placed in an inert matrix and is available in easily installable socks of various diameters. These socks can be stacked in wells screened across the entire thickness of the contaminated zone.

5.6.8 Natural Intrinsic Bioremediation

The basic concept behind "natural intrinsic bioremediation" is to allow the natural indigenous microorganisms to biodegrade the contaminant present in the groundwater. While natural

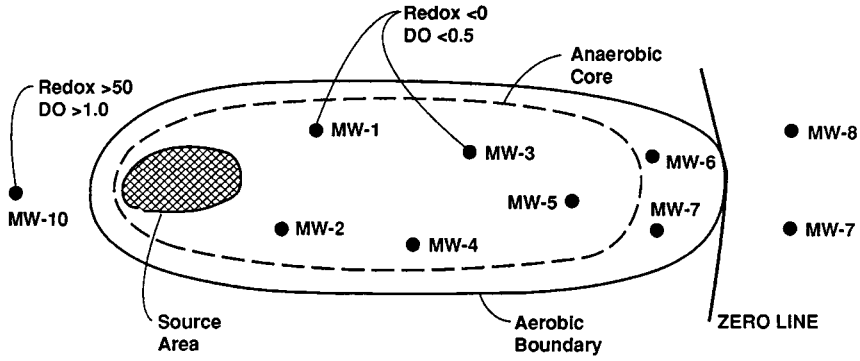


Figure 5.15 Concept of zero line.

attenuation processes include biodegradation, abiotic oxidation, hydrolysis, dispersion, dilution, sorption, and volatilization, intrinsic bioremediation is the primary mechanism for the attenuation of biodegradable contaminants. Intrinsic bioremediation, abiotic oxidation, and hydrolysis are the only attenuation mechanisms that destroy the contaminants to innocuous end products.

The use of intrinsic bioremediation as part of the site remediation strategy can significantly reduce cleanup costs. Intrinsic bioremediation is not a "no action" alternative. Implementation of a natural bioremediation system differs from conventional techniques, such that the contaminated plume is allowed to remain contaminated. Acceptance of natural bioremediation as a remediation alternative will be greatly enhanced if a *zero line* can be established (Figure 5.15).

The definition of the zero line (in plan view) is the location of a vertical plane in which the rate of natural degradation of contaminants exceeds the mass flux rate of contaminants. In the absence of any new release of contaminants, the zero line will not be a fixed, stationary line. Due to the dynamic natural attenuation processes, this line will be shifting toward the source area, thus resulting in a gradual shrinkage of the contaminant plume.

Existence of a zero line can be inferred by evaluating groundwater quality data over a period of time. Three to four rounds of sampling data collected over a period of time may indicate the existence of the zero line. However, the credibility of the argument will be greatly enhanced by providing supporting biogeochemical evidence collected from within and outside the plume. The data collected will indicate, in most cases where petroleum contamination is present, four distinct zones of biogeochemical dynamics: (1) the heart of the plume, (2) an anaerobic zone, (3) an aerobic zone, and (4) a remediated zone (Figure 5.16).

As noted in previous sections, various biodegradation pathways will take place in these four zones. Almost all dissolved petroleum hydrocarbons are biodegradable under aerobic conditions, where microorganisms utilize O_2 as the electron acceptor and the contaminants as the substrate for their growth and energy. When oxygen supply is depleted and nitrate is present, facultative anaerobic microorganisms will utilize NO_3^- as the electron acceptor. Once the available oxygen and nitrate are depleted, microorganisms may use oxidized ferric ion (Fe(III)) as an electron acceptor. When the redox conditions are further reduced (near the source area due to the abundance of contaminant mass), sulfate may act as the electron acceptor. Under significantly lower redox conditions (within the heart of the plume), methanogenic conditions will exist and the microorganisms can degrade the petroleum contaminants using water as the electron acceptor.

5.6.8.1 Concept of Bio-Buffering

Intrinsic bioremediation can use a wide range of electron acceptors under varying redox conditions. The biochemical reactions facilitated by these electron acceptors fall into two categories:

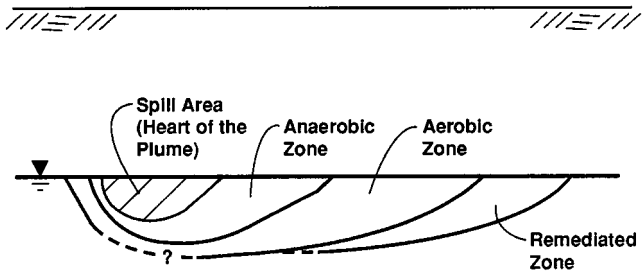


Figure 5.16 Four different zones depicting the natural intrinsic bioremedation process.

1. Relatively fast transformations that involve the use of O_2 and NO_3^-.
2. Relatively slow transformations that involve the reduction of Fe(III), SO_4^{2-}, and methanogenesis using H_2O.

The first reactions to occur are nearly instantaneous. Once the O_2 and NO_3^- are depleted and the environment turns more anaerobic, the slower reactions will begin. It is worth noting that even in a reducing environment, multiple reactions occur simultaneously, including the continuing reduction of O_2 owing to the replenishment of all electron acceptors by inflowing groundwater.

The concept of *bio-buffering* is based on the premise that the degradative capacity of the aquifer is a lot more than the available DO in the system. Bio-buffering also could be defined as the stability of the assimilative capacity of the natural system in response to the introduction of the contaminant mass flux into the aquifer. Among all the electron acceptors, O_2 and CO_2 are the most readily available, due to natural recharge processes and aquifer geochemistry. Sulfate, iron, and manganese also occur naturally, but are dependent on site minerology. Sulfate may be also introduced by manmade activities. The predominant sources of nitrate are anthropogenic activities such as agricultural fertilization.

Estimation of the assimilative capacity of benzene in an intrinsic bioremediation system is presented in the next few steps.

Aerobic Oxidation:

$$7.5O_2 + C_6H_6 \rightarrow 6CO_2 + 3H_2O$$

Mass ratio of O_2 to C_6H_6 = 3.1:1

0.32 mg/l benzene degraded per 1 mg/l of O_2 consumed

If the background DO concentration is 4.0 mg/l,

$$\text{assimilative capacity (aerobic biodegradation)} = \frac{0.32}{1} \times 4$$
$$= 1.28 \text{ mg/l}$$

Denitrification:

$$6NO_3^- + 6H^+ + C_6H_6 \rightarrow 6CO_2 + 6H_2O + N_2$$

Mass ratio of NO_3^- to C_6H_6 = 4.8:1

0.21 mg/l benzene degraded per 1 mg/l of NO_3^- consumed

If the background NO_3^- concentration is 12 mg/l,

$$\text{assimilative capacity (denitrification)} = \frac{0.21}{1} \times 12$$
$$= 2.52 \text{ mg/l}$$

Iron Reduction:

$$60H^{+} + 30Fe(OH)_3 + C_6H_6 \rightarrow 6CO_2 + 30Fe^{2+} + 78H_2O$$

Mass ratio of $Fe(OH)_3$ to C_6H_6 = 41:1

Mass ratio of Fe^{2+} produced to C_6H_6 degraded = 15.7:1

0.045 mg/l of benzene degraded per 1 mg/l of Fe^{2+} produced

If the background Fe^{2+} concentration is 25 mg/l,

$$\text{assimilative capacity (Iron)} = \frac{0.045}{1} \times 25$$
$$= 1.125 \text{ mg/l}$$

Sulfate Reduction:

$$7.5H^{+} + 3.75SO_4^{2-} + C_6H_6 \rightarrow 6CO_2 + 3.75H_2S + 3H_2O$$

Mass ratio of SO_4^{2-} to C_6H_6 = 4.6:1

0.22 mg/l benzene degraded per 1 mg/l of sulfate consumed

If background SO_4^{2-} concentration is 60 mg/l,

$$\text{assimilative capacity (sulfate reduction)} = \frac{0.22}{1} \times 60$$
$$= 13.2 \text{ mg/l}$$

Methanogenesis:

$$4.5H_2O + C_6H_6 \rightarrow 2.25CO_2 + 3.75CH_4$$

Mass ratio of CH_4 produced to C_6H_6 = 0.8:1

1.3 mg/l benzene degraded per 1 mg/l of CH_4 produced

If background methane concentration is 0 mg/l and

measured methane concentration is 4.0 mg/l,

$$\text{assimilative capacity (methanogenesis)} = \frac{1.3}{1} \times 4.0$$
$$= 5.2 \text{ mg/l}$$

5.6.8.2 Evaluation of Natural Intrinsic Bioremediation

Evaluation of natural intrinsic bioremediation can be performed by collecting and analyzing site-wide groundwater quality data with the following objectives:

- Documented loss of contaminant mass at the field scale.
- Biogeochemical indicator trends.
- Laboratory confirmation of microbial activity.

Collection of an adequate database during the site characterization process, over a period of time, is an important step in documenting intrinsic bioremediation. At a minimum, the site characterization phase should provide data on the location and extent of contaminant sources; the location, extent, and concentration of dissolved-phase contamination; geologic information on the type of soil distribution; hydrogeologic parameters such as hydraulic conductivity and hydraulic gradients; and groundwater biogeochemical data.[35–36]

Biogeochemical trends can be established by collecting groundwater samples and analyzing for the following parameters: dissolved oxygen (DO), redox potential, pH, temperature, conductivity, alkalinity, nitrate, sulfate, sulfide, ferrous iron, carbon dioxide, methane, and chloride, in addition to the contaminants.[36] The extent and distribution (vertical and horizontal) of contamination and electron acceptor and metabolic by-product concentrations are important in documenting the occurrence of intrinsic bioremediation.

If the dissolved oxygen concentration levels within the contamination plume are below background levels, it is an indication of aerobic biodegration at those locations. Similarly, nitrate and sulfate concentrations below background levels in the plume are indications of anaerobic biodegradation through denitrification and sulfate reduction. Presence of nitrite and H_2S in the plume will further enhance the evidence of denitrification and sulfate reduction. Furthermore, elevated concentrations of metabolic by-products such as ferrous ion and methane will indicate the occurrence of Fe(III) reduction and methanogenesis inside the plume. Contour maps should be developed to provide clearly visible trends of these processes inside the contamination plume.

Significant quantitative differences in the concentration levels of the various electron acceptors and metabolic by-products will be more than sufficient, in more cases, to claim the occurrence of natural intrinsic bioremediation. However, estimating the rate constants of contaminant degradation will further support the selection of this alternative as the preferred remediation method.

First-order rate constants can be calculated by the following equation:

$$\text{Rate} = \frac{\ln (\text{highest concentration downgradient}/\text{highest concentration upgradient})}{\text{distance traveled}/\text{plume velocity}}$$

It should be noted that the concentration at the downgradient location should be corrected for dilution. When estimating the plume velocity, the retardation factor of the contaminants considered should be taken into account.

Laboratory confirmation of microbial activity can vary from enumerating the microbial population to performing full-blown microcosm studies. Respirometric studies of the groundwater samples also can be performed in the laboratory.

Intrinsic bioremediation of CAHs should be evaluated differently than that of petroleum hydrocarbons. Since most of the metabolic pathways are induced by cometabolic mechanisms, presence of primary organic substrates and acclimatization of indigenous microorganisms will play significant role in natural bioremediation. Mineralization and nonmineralization pathways and accumulation of metabolic intermediates should be taken into serious consideration.

Starting concentration of the target contaminants, availability and concentration of electron acceptors, and presence of native organic compounds will all play a significant role in intrinsic bioremediation of chlorinated compounds.

Under optimal conditions, natural intrinsic bioremediation should be capable of completely containing a dissolved hydrocarbon plume. While there are an increasing number of well-documented cases where this has occurred, there is a great deal of anecdotal evidence that suggests this is possible.

One of the interesting questions that is currently being investigated by researchers is whether it may be possible to complete a mass balance on the supply of electron donors and electron acceptors at a given site. This question is complicated because of sampling and field data collection limitations. Other complicating factors involve the temporal nature of the distributions of electron acceptors and donors.

5.7 BIOMODELING

Groundwater transport and fate models have traditionally focused on modeling advection, dispersion, and sorption as three main attenuation mechanisms in groundwater. A fourth key variable that impacts the fate of contaminants is biodegradation. Development of biodegradation models is not simple, because of the complex nature of microbial kinetics, the lack of accurate field data, and the lack of robust numerical schemes that can simulate the physical, chemical, and biological processes accurately. Several researchers have developed groundwater biodegradation models.[37] The main approaches used for modeling biodegradation kinetics are

- first-order degradation models
- biofilm models (including kinetic expressions)
- instantaneous reaction models, and
- dual-substrate Monod models.

One of the most popular models used in biomodeling of petroleum hydrocarbons degradation is the BIOPLUME model. This model incorporates a system of equations to simulate the simultaneous growth, decay, and transport of microorganisms combined with the transport and removal of hydrocarbons and oxygen.[37] This model was later expanded and extended and released as BIOPLUME II.

BIOPLUME II model included two expressions for simulation of biodegradation: (1) first-order decay of the contaminants, and (2) aerobic decay based on the dissolved oxygen concentrations present in the groundwater. BIOPLUME II relied on the same concept used in BIOPLUME I, which showed that when biodegradation occurs rapidly relative to groundwater velocities, the process can be assumed to occur instantaneously. In other words, the rate of reaction can be neglected, and the biodegradation of contaminants using oxygen as an electron acceptor is based solely on the stoichiometry of the chemical reaction.[35,37]

BIOPLUME II incorporated three different sources of oxygen: (1) background levels of oxygen prior to contamination, (2) oxygen supply from external sources (H_2O_2 or air injection), and (3) dissolved oxygen replenished by the moving groundwater. Despite its popularity, BIOPLUME II has two main limitations: (1) it does not account for slowly degrading compounds, and (2) it does not allow for simulating anaerobic processes.

BIOPLUME III, in contrast, simulates the transport and fate of six components in groundwater: contaminant, DO, NO_3^-, Fe, SO_4^{2-}, and CO_2. The biodegradation model assumed in BIOPLUME III is the sequential utilization of electron acceptors.[38]

$$O_2 \rightarrow NO_3^- \rightarrow Fe^{3+},\ SO_4^{2-} \rightarrow CO_2$$

For each of the electron acceptors, a number of biodegradation kinetic expressions such as first-order decay and instantaneous or Monod kinetics can be selected. Except for the aerobic and denitrification pathways, instantaneous reaction kinetics should be avoided.

5.8 PRIMARY KNOWLEDGE GAPS

Knowledge gaps include both those items that are not understood well and the myriad of information known by practitioners that has not been disseminated to the general audience.[39] As the published reports are getting more and more detailed, increasingly specific questions are being asked.

- Identification of the cause and effect of some unexpected results obtained in some field demonstration studies.
- Better scale-up of laboratory results to field performance.
- Evaluation of suitable habitats, nutritional requirements, lag times, and degradation rates (in the field) for various contaminants.
- Metabolic pathways of many contaminants of concern which are still unknown.
- Optimization of environmental conditions, and stimulation of favorable growth conditions under site-specific variations.
- Effect of NAPLs.
- *In situ* methods for monitoring process efficiency.
- Mass balance of electron donors and acceptors within a given system.
- Impact on aquifer permeability due to enhanced bioremediation.
- Bioavailability of higher molecular weight hydrocarbons. Enhancing bioremediation in low permeable environments.

REFERENCES

1. Alexander, M., *Biodegradation and Bioremediation*, Academic Press, New York, 1994.
2. Cookson, J. T., *Bioremediation Engineering: Design and Application*, McGraw-Hill, New York, 1994.
3. Bitton, L. N., Microbial degradation of aliphatic hydrocarbons, in *Microbial Degradation of Organic Compounds*, Gibson, D. T., Ed., Marcel Dekker, New York, 1984.
4. Wackeff, L. D., Brusseau, G. A., Householder, S. R., and Hansen, R. S., Survey of microbial oxygynases: trichloroethylene degradation by propane-oxidizing bacteria, *Appl. Environ. Microbiol.*, 55, 2960, 1989.
5. Doelman, P., Microbiology of soil and sediments, in *Biogeodynamics of Pollutants in Soils and Sediments*, Salomons, W. and Stigliani, W. M., Eds., Springer, Berlin, 1995.
6. Salamons, W., Long term strategies for handling contaminated sites and large scale areas, in *Biogeodynamics of Pollutants in Soils and Sediments*, Salomons, W. and Stigliani, W. M., Eds., Springer, Berlin, 1995.
7. Reinhard, M., In situ bioremediation technologies for petroleum derived hydrocarbons based on alternate electron acceptors (other than molecular oxygen), in *Handbook of Bioremediation*, Norris, R. D., et al., Lewis Publishers, Boca Raton, FL, 1994.
8. Grbić-Galić, D., Microbial Degradation of Homocyclic and Heterocyclic Aromatic Hydrocarbons under Anaerobic Conditions, unpublished report, Department of Civil Engineering, Environmental Engineering and Science, Stanford University, Palo Alto, CA, 1990.
9. McKenna, E., Biodegradation of Polynuclear Aromatic Hydrocarbon Pollutants by Soil and Water Microorganisms, Final Report, Project No. A-073-ILL, University of Illinois, Water Resources Center, Urbana, IL, 1979.
10. Dhawale, S. W., Dhawale, S. S., and Dean-Ross, D., Degradation of phenanthrene by *Phanarochaete chrysosporium* occurs under ligninolytic as well as nonligninolytic conditions, *Appl. Environ. Microbiol.*, 53, 3000, 1992.
11. Semprini, L., Grbić-Galić, D., McCarty, P. L., and Roberts, P. V., Methodologies for Evaluating In Situ Bioremediation of Chlorinated Solvents, U.S. Environmental Protection Agency, EPA/600/R-92.042, 1992.
12. McCarty, P. L. and Semprini, L., Groundwater treatment for chlorinated solvents, in *Handbook of Bioremediation*, Norris, R. D., et al., Eds., Lewis Publishers, Boca Raton, FL, 1994.
13. Vogel, T. M., Criddle, C. S., and McCarty, P. L., Transformations of halogenated aliphatic compounds, *Environ. Sci. Technol.*, 21(8), 722, 1987.
14. Bouwer, E. J., Bioremediation of chlorinated solvents using alternate electron acceptors, in *Handbook of Bioremediation*, Norris, R. D., et al., Lewis Publishers, Boca Raton, FL, 1994.
15. Bouwer, E. J., Rithmann, B. E., and McCarty, P. L., Anaerobic degradation of halogenated 1- and 2-carbon organic compounds, *Environ. Sci. Technol.*, 15, 596, 1981.

16. Bouwer, E. J. and Wright, J. P., Transformations of trace halogenated aliphatics in anoxic biofilm columns, *J. Contam. Hydro.*, 2, 155, 1988.
17. Wilson, J. T. and Wilson, B. H., Biotransformation of trichloroethylene in soil, *Appl. Environ. Microbiol.*, 49(1), 242, 1988.
18. Tsien, H. C., Bousseau, A., Hanson, R. S., and Wackeff, L. P., Biodegradation of trichloroethylene by *Methylosinus trichosporium*, *Appl. Environ. Microbiol.*, 55, 3155, 1989.
19. Palumbo, A. V., Eng, W., Boerman, P. A., Strandberg, G. W., Donaldson, T. L., and Herber, S. E., Effects of diverse organic contaminants on trichloroethylene degradation by methanotrophic bacteria and methane-utilizing consortia, in *On Site Bioreclamation Processes for Xenobiotic and Hydrocarbon Treatment*, Hinchee, R. E. and Offenbuttel, R. F., Eds., Butterworth-Heinemann, Stoneham, MA, 1991.
20. Young, J. C., Anaerobic degradation of aromatic compounds, in *Microbial Degradation of Organic Compounds*, Gibson, D. T., Ed., Marcel Dekker, NY, 1984.
21. Zhang, X. and Wiegel, J., Sequential anaerobic degradation of 2,4-dichlorophenol in freshwater sediments, *Appl. Environ. Microbiol.*, 58, 2993, 1990.
22. Brunner, W., Sutherland, F. H., and Focht, D.-D., Enhanced biodegradation of polychlorinated biphenyls in soil by analog enrichment and bacterial innoculation, *J. Environ. Qual.*, 14, 324, 1985.
23. Rhee, G. Y., Sokul, R. C., Bush, B., and Bethoney, C. M., Long term study of the anaerobic dechlorination of Aroclor 1254 with and without biphenyl enrichment, *Environ. Sci. Technol.*, 27, 714, 1993.
24. Luey, J., Brouns, T. M., and Elliott, M. L., Biodegradation of Hazardous Waste Using White Pot Fungus: Project Planning and Concept Development Document, report prepared by Pacific Northwest Laboratory to U.S. Department of Energy, Richland, Washington, 1990.
25. Simkins, S. M. and Alexander, M., Models for mineralization kinetics with the variables of substrate concentration and population density, *Appl. Environ. Microbiol.*, 47, 1299, 1984.
26. Stanier, R. Y., Ingraham, J. L., Wheelis, M. L., and Painter, P. R., *The Microbial World*, 5th ed., Prentice-Hall, Englewood Cliffs, NJ, 1986.
27. Lee, M. D., Thomas, J. M., Border, R. C., Bedient, P. B., Ward, C. H., and Wilson, J. T., *CRC Critical Reviews in Environmental Control — Biorestoration of Aquifers Contaminated with Organic Compounds*, Report, Rice University, vol. 18, 1988.
28. Jamison, V. W., Raymond, R. L., and Hudson, J. O., Biodegradation of high octane gasoline in groundwater, *Devel. Indust. Microbiol.*, 16, 305, 1976.
29. Berry-Spark, K. and Barker, J. F., Nitrate remediation of gasoline contaminated groundwaters: results of a controlled field experiment, in *Proceedings of the NWWA/API Conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection and Restoration*, Houston, TX, Nov. 1987.
30. Hutchins, S. R., Miller, D. E., Beck, F. P., Thomas, A., Williams, S. E., and Willis, G. D., Nitrate based bioremediation of JP-4 jet fuel: Pilot scale demonstration, in *Applied Bioremediation of Petroleum Hydrocarbons*, Hinchee, R. E., Kittel, J. A., and Reisinger, H. J., Eds., Battelle Press, Columbus, OH, 1995.
31. Michelsen, D. L. and Lofti, M., Oxygen microbubble injection for in situ bioremediation: Possible field scenario, in *Biological Processes: Innovative Waste Treatment Technology Series*, vol. 3., Freeman, H. M. and Sferra, P. R., Eds., Technomic Publishing, Lancaster, PA, 1993.
32. Saaty, R. P., Showalter, E. W., and Booth, S. R., Cost effectiveness of in situ bioremediation at Savannah River, in *Bioremediation of Chlorinated Solvents*, Hinchee, R. E., Leeson, A., and Semprini, L., Eds., Battelle Press, Columbus, OH, 1995.
33. Norris, R. D., personal communication, 1995.
34. Ochs, L. D., personal communication, 1995.
35. Wilson, B. H., Wilson, J. T., Kampbell, D. H., and Bledsoe, B. E., *Traverse City: Geochemistry and Intrinsic Bioremediation of BTEX Compounds*, U.S. Environmental Protection Agency, USEPA/540/R-94/515, 1994.
36. Weidemeier, T. H., Wilson, J. T., Miller, R. N., and Kampbell, D. H., United States Air Force guidelines for successfully supporting intrinsic remediation with an example from Hill Air Force Base, *National Water Well Association/American Petroleum Institute Outdoor Action Conference*, Las Vegas, NV, 1994.
37. Bedient, P. B. and Rifai, H. S., Modeling in situ bioremediation, in *In Situ Bioremediation: When Does it Work*, National Research Council, National Academy Press, Washington, DC, 1993.

38. Rifai, H. S., Newell, C. J., Miller, R. N., Tiffinder, S., and Rounsaville, M., Simulation of natural attenuation with multiple electron acceptors, in *Intrinsic Bioremediation*, Hinchee, R. E., Wilson, J. T., and Downey, D. C., Eds., Battelle Press, Columbus, OH, 1995.
39. Norris, R. D., In situ bioremediation of soils and groundwater contaminated with petroleum hydrocarbons, in *Handbook of Bioremediation*, Norris, R. D., et al., Eds., Lewis Publishers, Boca Raton, FL, 1994.