Water Environment Federation
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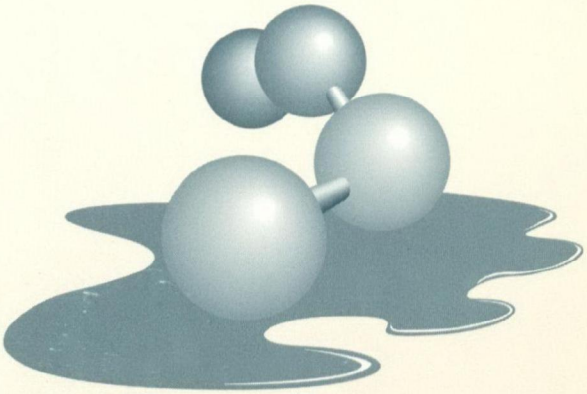
IN-SITU BIOREMEDIATION

OF

CONTAMINATED SUBSURFACE MEDIA



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SECTION 1
INTRODUCTION

1.1 Background

Due to high costs associated with excavation and incineration for treating hazardous wastes and materials contaminated with those wastes, alternative innovative technologies have been sought. One innovative technology, in-situ bioremediation of contaminants in the subsurface, has been the focus of considerable research in the past decade. A variety of approaches have been developed, differing mainly in the mechanism whereby essential nutrients and electron acceptors are delivered. Factors such as site hydrogeology and contaminant nature and distribution often control the success of in-situ bioremediation efforts.

The use of biooxidation for environmental purposes has been practiced for decades, with biological processes used in wastewater treatment since the early 1900s. Activated sludge and fixed-film growth systems are commonly used for treating municipal wastewater and industrial wastes. This use of biological degradation of organic compounds has generated a wide body of information regarding biodegradability of specific compounds and classes of chemicals, nutrient and electron acceptor requirements, and oxidation mechanisms. Land treatment processes for municipal wastewater and sludges, as well as petroleum refinery wastes, have also been practiced for several decades and have generated additional information on nutrient requirements, degradation rates, and other critical parameters affecting biological oxidation (Overcash and Pal, 1979).

In the 1970s, several studies sponsored by the American Petroleum Institute were conducted using the method developed by Richard L. Raymond, then at Sun Tech.,to biologically degrade hydrocarbons in aquifers (Bauman, 1991). This method involved the recovery of ground water, treatment using an air stripper tower and subsequent reinjection following amendment with nitrogen and phosphorus sources (Raymond et al., 1976). Many of these early tests were conducted prior to the enactment of federal and state mandated clean-up levels. As a result, these tests demonstrated that in-situ bioremediation could reduce the levels of petroleum hydrocarbons in an aquifer, but did not document an ability to reach ground-water quality standards in today's regulatory environment.

In the mid 1980s, there were few companies with experience in bioremediation of aquifers or soils. Since that time, many companies have utilized bioremediation technologies, although claims of experience are frequently overstated. Acceptance of in-situ bioremediation as a remediation technology by the public and various regulatory agencies has been generally favorable over the last seven or eight years and has improved significantly over the last two or three years with the support of the U.S. EPA, many state agencies, and favorable publicity in trade journals and the popular press. As an in-situ technology that is viewed as a natural process resulting in destruction rather than relocation of contaminants, in-situ bioremediation meets many of the objectives of state and federal agencies.

1.2 Scope

It is the intent of this report to provide the reader with a detailed background of the technologies available for in-situ bioremediation of contaminated soil and ground water. The document has been prepared for scientists, consultants, regulatory personnel, and others who are associated in some way with the restoration of soil and ground water at hazardous waste sites. The presentation provides the most

recent scientific understanding of the processes involved with bioremediation of soil and ground-water, as well as a definition of the state-of-the-art of these technologies with respect to circumstances of their applicability and their limitations.

A number of bioremediation technologies are discussed, along with the biological processes driving those technologies. In addition to discussions and examples of developed technologies, the report also provides insights to emerging technologies which are at the research level of formation, ranging from theoretical concepts, through bench scale inquiries, to limited field-scale investigations. Although a wide range of contaminants are potentially biodegradable, bioremediation systems have been most successfully applied to petroleum hydrocarbons (fuels and refinery wastes), wood preserving wastes (creosote), and chlorinated solvents (TCE). Therefore, the major focus of this report is limited to those contaminant groups.

SECTION 2
THE SUBSURFACE ENVIRONMENT

The applicability and success of in-situ bioremediation processes are primarily determined by the geology and hydrology of sites. The physical and chemical nature of contaminants, as well as their distribution in the subsurface, are also critical in determining whether in-situ bioremediation is successful. In addition, the nature and performance of existing microbial populations are vital to successful application of bioremediation technologies. Therefore, an understanding of key subsurface elements impacting biodegradation processes is presented in the following section.

The physical subsurface environment can be visualized as having two major compartments. These include: (1) a stationary, solid phase; and (2) a fluid or transient phase comprised of liquids and/or gas in the void spaces between the solids (**Figure 1**). The solid phase consists of inorganic materials such as clay, silt, sand, and gravel; and organic materials such as humic and fulvic acids. The physical and chemical properties of the solid phase control the transport and transformation characteristics of contaminants in this environment, as well as our ability to apply remediation activities. The void, or pore, spaces are the primary pathway for movement of the fluid phases and, therefore, are the pathway for contaminant transport in the subsurface environment. These spaces may be filled with either air (unsaturated) or water (saturated). If non-aqueous phase liquid (NAPL) contaminants (such as gasoline) are present, the pore space may be occupied by a mixture of air, water, and NAPL. Thus, contaminants in the subsurface may partition between four major compartments (Huling and Weaver, 1991): (1) air phase—vapor in the pore spaces; (2) adsorbed phase—sorbed to subsurface solids; (3) aqueous phase—dissolved in water; and (4) NAPL phase—non-aqueous liquids. These compartments are illustrated in **Figure 2**.

2.1 Geology/Hydrology

The geology and hydrology of the subsurface at a contaminated site play critical roles in determining contaminant distribution; further, they determine whether or not in-situ bioremediation is feasible.

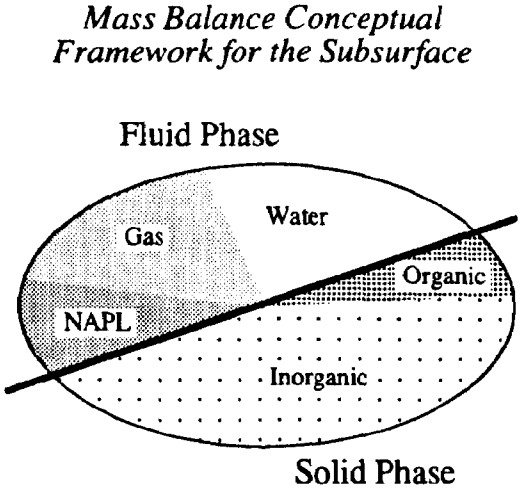

Figure 1. Conceptual framework for major subsurface compartments (USEPA, 1992a).

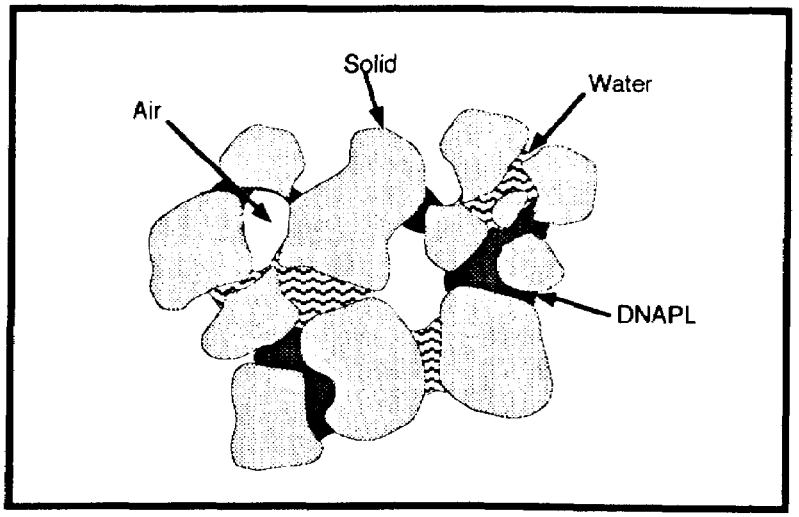

Figure 2. **Subsurface phases into which contaminants may partition (Huling and Weaver, 1991).**

2.1.1 Porosity, Permeability, and Hydraulic Conductivity

Two major parameters which describe the subsurface in terms of water and contaminant transport are **porosity** and **permeability**. Porosity refers to the amount of pore, or void, space present in a specific volume of subsurface material. This parameter indicates the amount of storage that is available in soil or aquifer materials as a function of particle size and texture. The porosities of several subsurface materials are given in **Figure 3**. For example, clay can hold more water and chemicals than gravel because of its greater porosity. **Permeability**, on the other hand, indicates the relative ease with which fluids move through subsurface material, including water, with the nutrients and oxygen required for enhanced in-situ bioremediation. Permeability is a function of the **pore size**. Materials with smaller pore sizes, although more porous, often exhibit low permeability. As shown in **Figure 4**, clay has much more porosity than gravel, yet gravels can be orders of magnitude more permeable than clay. **Hydraulic conductivity** is a commonly used hydrogeologic measure of permeability and is often a limiting factor in applying in-situ bioremediation. Contaminated subsurface materials with high porosity (storage) and low hydraulic conductivities are poor candidates for in-situ bioremediation.

2.2 Subsurface Heterogeneity

A frequently overlooked characteristic of subsurface environments is the inherent variability, or heterogeneity, of various layers. During site characterizations, a 'conceptual model' of the subsurface is developed, often based on relatively little data. These models often overlook the presence of silt or clay 'lenses' which may not have been detected during geological investigations. Since ground water and soil vapors will follow the path of least resistance in response to force (hydraulic or pneumatic gradients), areas in the subsurface with high permeability will become preferential flowpaths. Regions in the subsurface with lowest permeabilities, such as clays and silts, will remain contaminated. Even geologic layers differing only in grain sizes, such as a fine sand layer below a coarse sand layer, may have significantly different permeability. These subsurface heterogeneities play critical roles in contaminant transport as well as in-situ remediation.

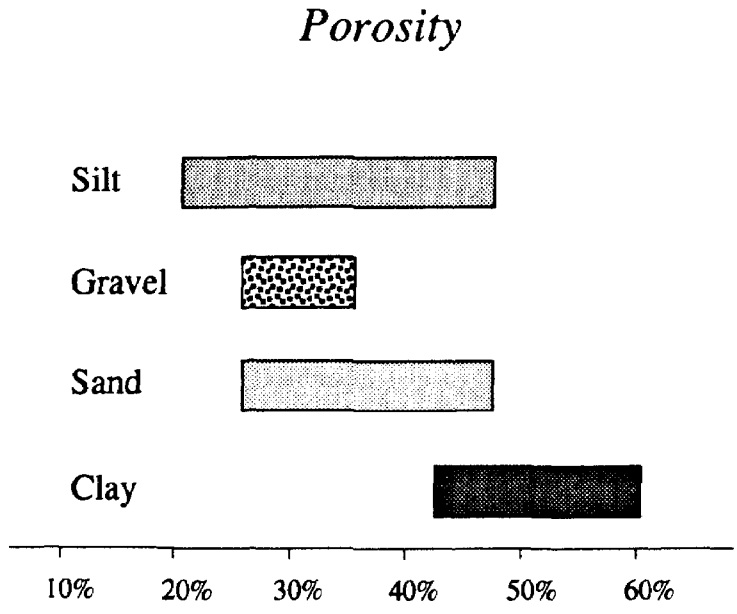

Figure 3. Porosity of subsurface materials (USEPA, 1992a).

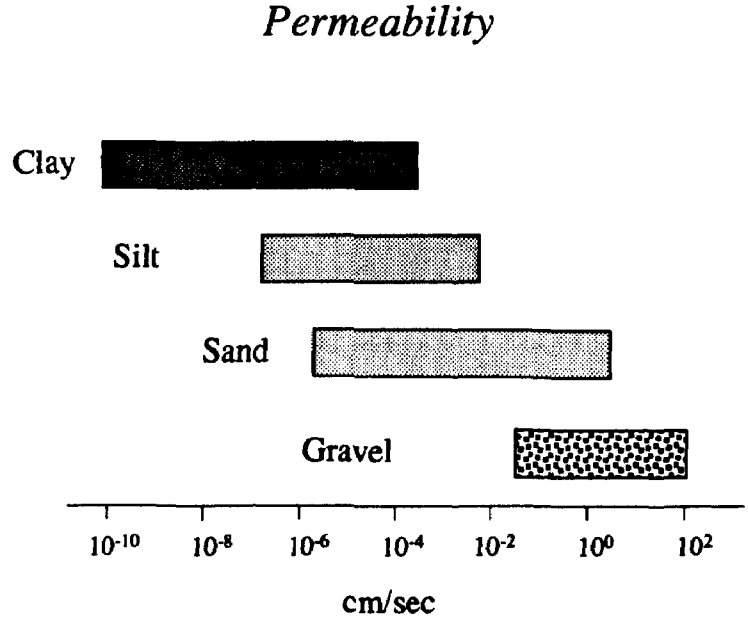

Figure 4. Permeability of subsurface materials (USEPA, 1992a).

2.3 Contaminant Distribution

It is important to understand where contaminants are distributed among the various subsurface compartments shown in Figure 2. This phase partitioning of contaminants is dependent upon a number of factors, including the physical/chemical nature of the contaminants as well as that of the subsurface environment. This distribution is exemplified in **Figure 5,** where contaminants are shown to be associated with the vapor phase in the unsaturated zone, a residual phase, or dissolved in ground water.

Four major characteristics determine which subsurface compartment a contaminant will be most likely to partition into and, subsequently, to what extent in-situ bioremediation will be applicable to the contaminant. These are discussed briefly below:

2.3.1 Solubility

Solubility in water plays a critical role in transport and biodegradation of contaminants. Those contaminants which are very water soluble will partition to the aqueous compartment in the subsurface. Contaminants in the water phase, both in bulk liquid moving through pores (saturated) and in water films surrounding particles (unsaturated), are most exposed to action of subsurface microbial communities. Because of this, more water soluble contaminants are generally more biodegradable.

2.3.2 Sorption

Contaminants may partition to the subsurface solid phase. This process is referred to as **sorption.** Sorption results when the contaminant interacts with either natural organic matter, usually humic and/or fulvic acids, associated with soils or aquifer materials, or directly with the mineral surface. The degree and 'tightness,' or reversibility, of sorption depends on the chemical structure of both the contaminant and the organic matter, as well as the amount of organic matter present. The overall effect of sorption is to

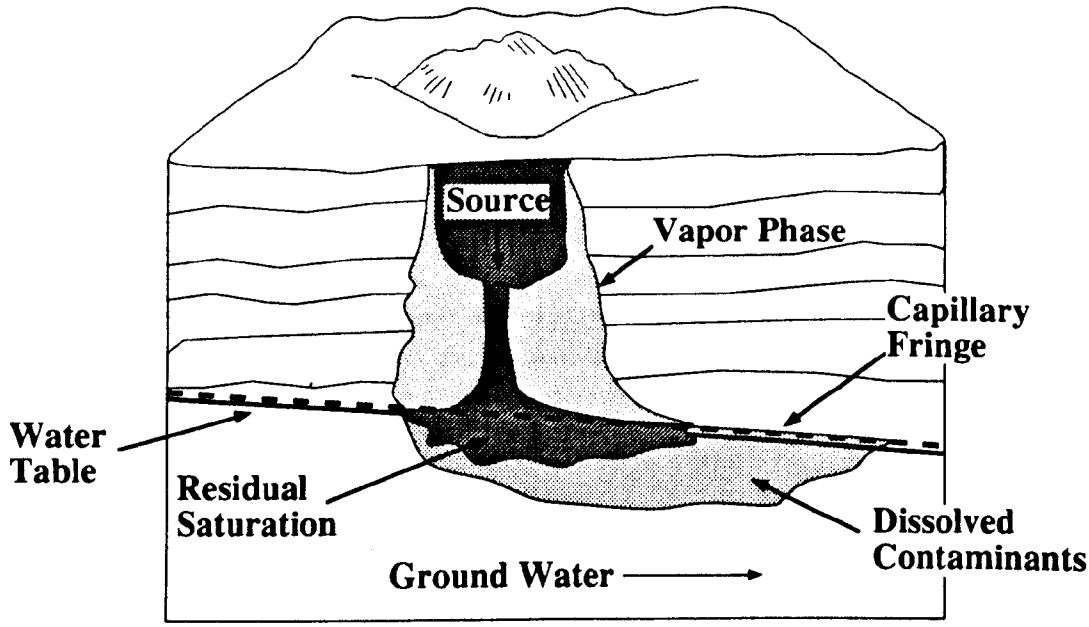

Figure 5. Possible contaminant locations in the subsurface (shaded areas).

retard the movement of contaminants in the subsurface. Contaminants which are tightly sorbed to subsurface materials such as clays are often not **bioavailable** and are resistant to biodegradation. Examples of such contaminants include high molecular weight polyaromatic hydrocarbons such as benzo (a) pyrene.

2.3.3 Volatility

Contaminants which have high vapor pressures partition into the air occupying the pore spaces between particles. Once in the gas phase, vapors may migrate in response to air pressure gradients or gravity, depending on the density of the vapor relative to air.

2.3.4 NAPLs

As noted above many contaminants do not mix with water and exist as distinct non-aqueous phase liquids (NAPLs) in the subsurface. These may be either lighter or heavier than water, thus resulting in the terms **LNAPL** (light non-aqueous phase liquids) and **DNAPL** (dense non-aqueous phase liquids). When NAPLs exist as a continuous body of immiscible phase, they migrate vertically as a bulk liquid, leaving behind isolated globules of material trapped in the pore spaces by capillary forces. This **residual saturation phase** remains as a significant continuous source of contamination to ground water. For example, gasoline trapped at residual saturation in an aquifer may occupy up to 50 percent of the pore space. Components of the gasoline such as benzene, toluene, and xylene partition or "bleed" into the transient water and vapor phases and therefore serve as long-term sources of contamination. If sufficient NAPL exists as bulk liquid, it will migrate to the water table. LNAPLs such as petroleum hydrocarbon fuels will spread out into a floating 'lens' on the water table. Fluctuations in the water table level may spread or 'smear' the lens, resulting in a greater volume of contaminated subsurface material over time (**Figure 6**). DNAPLs such as chlorinated solvents and creosote will continue to migrate downward through the aquifer until a relatively impermeable layer is reached, leaving behind more isolated globules as residual saturation. **Free-phase product** may collect as pools or ponds in depressions on top of these impermeable layers. Clay or silt lenses often act as such impermeable layers, leading to unpredictable horizontal migration if the surface is tilted. The DNAPL may then follow the slope of the surface until the lens ends, after which it continues downward to the next impermeable barrier. Typical patterns of DNAPL migration are shown in **Figures 7 and 8**. Finding and quantifying DNAPLs in the subsurface is difficult, and remediation technologies are just emerging.

2.4 Critical Site Limitations to Bioremediation

2.4.1 Hydraulic Conductivity

As noted above, permeability of the subsurface describes the ease with which fluids, either water or air, can be moved through contaminated soils and aquifers. Since water is often the primary mechanism for introducing amendments such as nutrients during in-situ bioremediation, successful application of the technologies depends on the ability to move water into and through the subsurface. Therefore, ground-water flow rate and flow paths are critical to the design and performance of in-situ bioremediation systems. The ground-water flow must be sufficient to deliver the required nutrients and oxygen (or other electron acceptors) according to the demand of the organisms, and the amended ground water should sweep the entire area requiring treatment. This is a critical point in that it is often the hydraulic conductivity of the ground-water system itself or the variability of the aquifer materials which limits the effectiveness of in-situ technologies or prevents its utility entirely. A suggested target for in-situ remediation technologies is a hydraulic conductivity of at least 10^{-4} cm/sec (100 ft/yr). Soils or

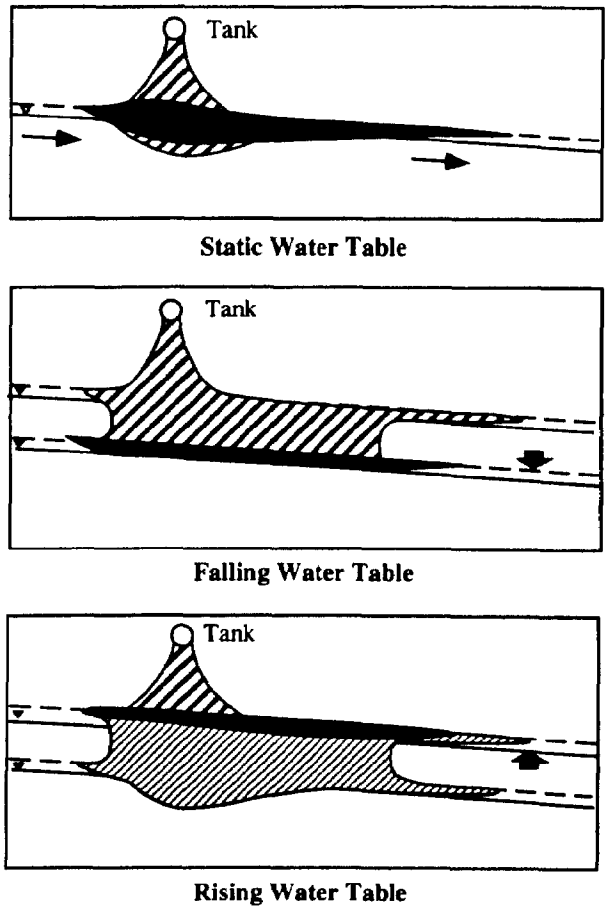

Figure 6. Smearing of hydrocarbon lens during water table fluctuations (API, 1989).

aquifers with hydraulic conductivities less than this value are poor candidates for in-situ bioremediation, due to extremely slow delivery rates of electron acceptors and nutrients.

2.4.2 Subsurface Heterogeneity

Subsurface heterogeneity can seriously limit the effectiveness of in-situ bioremediation. Discontinuous layers or lenses of clay and silt may serve as long term sources of contamination, due to their high storage and low permeability. Preferential ground water flow paths often develop in response to slight variations in permeability, resulting in only partial remediation of aquifers. Sites with a high degree of geological complexity are therefore often poor candidates for in-situ bioremediation.

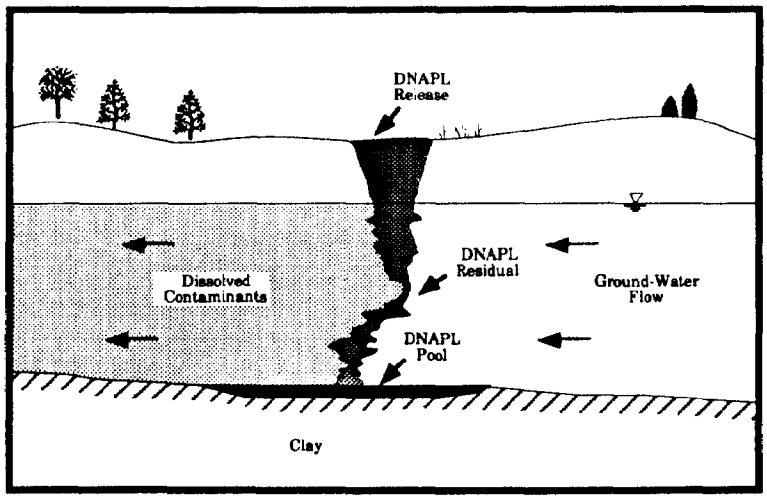

Figure 7. DNAPL migration patterns in homogeneous aquifers (Huling and Weaver, 1991).

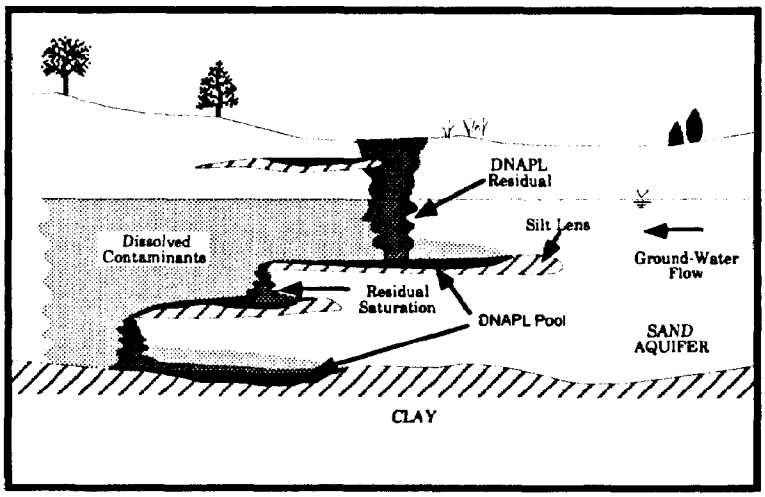

Figure 8. DNAPL migration patterns in heterogeneous aquifers (Huling and Weaver, 1991).

2.4.3 NAPLS and Residual Saturation

The presence of either LNAPL or DNAPL as free-phase product in the subsurface presents a major limitation to in-situ bioremediation systems for several reasons. First, if pore spaces are completely saturated with the non-aqueous phase, water is excluded. Therefore, ground water amended with nutrients and essential electron acceptors cannot come into contact with the bulk of the contamination, and bioremediation is limited. Second, although the rate of mass transfer of contaminants from the NAPL

phase to the ground water may be relatively slow, high enough concentrations of contaminants can develop in ground water near the NAPL to cause toxicity to microbes essential to the bioremediation process. Last, even if the first two limitations could be circumvented, the nutrient and electron acceptor requirements to biodegrade large quantities of contaminants would not be feasible. Therefore, every effort needs to be made to remove as much NAPL as possible before attempting bioremediation.

NAPLs present at residual saturation represent an often unrecognized source of contaminants. Historically, in-situ bioremediation addressed only contaminants dissolved in ground water; and many sites have been declared "clean" based on reduction of concentrations in ground water. However, NAPLs at residual saturation have much higher surface area in contact with ground water; and ground water can move much more freely through subsurface zones where pore spaces are not completely saturated with NAPL. Therefore, bioremediation systems should address not only the contaminant present in ground water, but also this significant remaining source.

SECTION 3
THE BIOREMEDIATION CONCEPT

3.1 Fundamental Principles

Biodegradation of contaminants by microbial populations occurs to some degree **without human intervention** in most ecosystems. These natural biodegradation mechanisms—along with abiotic processes such as hydrolysis, dispersion, volatilization, and sorption—are collectively know as "**natural attenuation.**" As illustrated in **Figure 9**, biodegradation of contaminants requires nutrients, especially nitrogen and phosphorous. In addition, microbial degradation of contaminants requires a **terminal electron acceptor**. Oxygen is the required electron acceptor in many populations of microbes degrading contaminants, and such populations are referred to as **aerobic**. In contrast, there are significant populations of microbes in the subsurface which utilize electron acceptors other than oxygen. Biological processes which occur in the absence of oxygen are referred to as **anaerobic**. There is considerable evidence that both of these general types of microbial metabolism are operative and potentially applicable in many contaminated subsurface ecosystems.

Biological treatment, whether of excavated soils, aquifer solids, or unsaturated subsurface materials, uses microorganisms to convert harmful chemical species to less harmful chemical species in order to effect remediation of a site or a portion of a site. The microorganisms are generally bacteria but can be fungi. The terms "natural attenuation," "natural bioremediation," and "passive bioremediation" are used somewhat interchangeably to describe the use of unassisted natural biodegradation processes for site remediation. Abiotic processes also contribute to this "intrinsic" attenuation capacity of the subsurface. Intrinsic bioremediation is therefore distinctly different from, and should not be confused with, technologies which actively adjust subsurface conditions to maximize biodegradation rates.

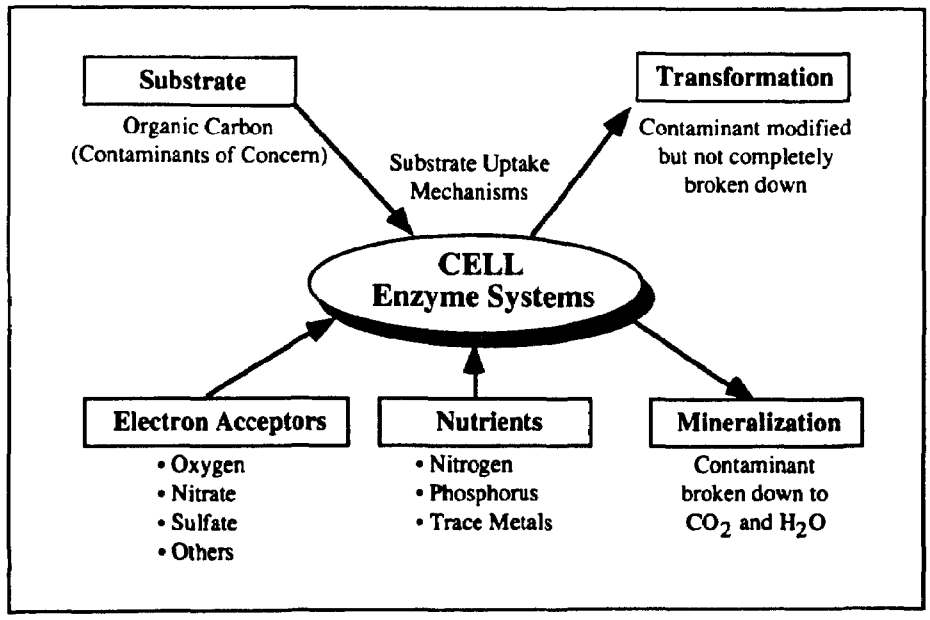

Figure 9. Concept overview: major components of biodegradation.

Engineered bioremediation refers to active processes designed to maximize biodegradation of contaminants through a variety of mechanisms, most involving addition of essential nutrients and electron acceptors. **In-situ bioremediation** refers to technologies designed to treat contaminated soils, ground water, or aquifer materials **in place**, with a minimum of excavation and other site disturbances.

3.2 Natural or "Intrinsic" Bioremediation

Microbial populations are capable of adapting to and degrading contaminants, and indigenous bacteria that can degrade a variety of organic compounds are present in nearly all subsurface materials (Borden, in press). The ability of microorganisms to degrade a wide variety of hydrocarbons is well known. In an early review, Zobell (1946) identified over 100 microbial species from 30 genera that could degrade some type of hydrocarbon. Since then, numerous studies have shown that hydrocarbon-degrading microorganisms are widespread in the environment and occur in fresh and salt water, soil, and ground water. Litchfield and Clark (1973) analyzed ground-water samples from 12 different aquifers throughout the United States that were contaminated with hydrocarbons. These workers found hydrocarbon-utilizing bacteria in all samples at densities up to 1.0×10^6 cells per ml. After a gasoline spill in Southern California, McKee et al. (1972) found 50,000 hydrocarbon degrading bacteria per ml or higher in samples from wells containing traces of gasoline, while a noncontaminated well had only 200 organisms per ml. Jamison et al. (1975) reported naturally occurring biodegradation of high octane gasoline in ground water. Research at sites contaminated with wood-preserving wastes (Lee and Ward, 1984; Wilson et al., 1985) demonstrated that an adapted population of creosote-degrading microorganisms was present within the contaminated zone, but not in the uncontaminated regions, of the aquifer. Other studies correlated creosote biodegradation with the availability of dissolved oxygen (Lee and Ward, 1984). In a more recent study, Ridgeway et al. (1990) identified 309 gasoline-degrading species of bacteria from a shallow coastal aquifer contaminated with unleaded gasoline.

Ongoing research has shown that an aquifer's intrinsic assimilative capacity depends on the metabolic capabilities of the native microorganisms, the aquifer hydrogeology and geochemistry, and the contaminants involved. In many aquifers, conditions will not be perfect for natural bioremediation; and less than optimal biodegradation will occur. The extent of aerobic biodegradation is controlled by the amount of contamination released, the rate of oxygen transfer into the subsurface, and the background oxygen content of the aquifer. When large amounts of contamination enter the subsurface, it overwhelms the capacity of an aquifer to assimilate them, and extensive contamination may persist for long times and distances downgradient from sources. When hydrogeologic conditions, such as clay confining layers or naturally-occurring organic deposits, reduce the rate of oxygen transfer into the subsurface, the assimilative capacity of the aquifer may be lower. Anaerobic biodegradation may be inhibited by low pH, low buffering capacity, absence of appropriate electron acceptors (nitrate, iron, etc.), or the presence of small amounts of oxygen. Heterogeneous conditions within the aquifer may prevent mixing and allow a portion of the plume to migrate rapidly. If this occurs, the extent of biodegradation may be less than would be expected for more uniform conditions.

3.2.1 Using Natural Biodegradation for Site Remediation

As the cost of performing site remediation continues to increase, interest in using "intrinsic attenuation" also has increased. Intrinsic bioremediation is capable of treating contaminants aerobically in the vadose zone, and at the margins of plumes (**Figure 10**) where oxygen is not limiting due to reoxygenation.

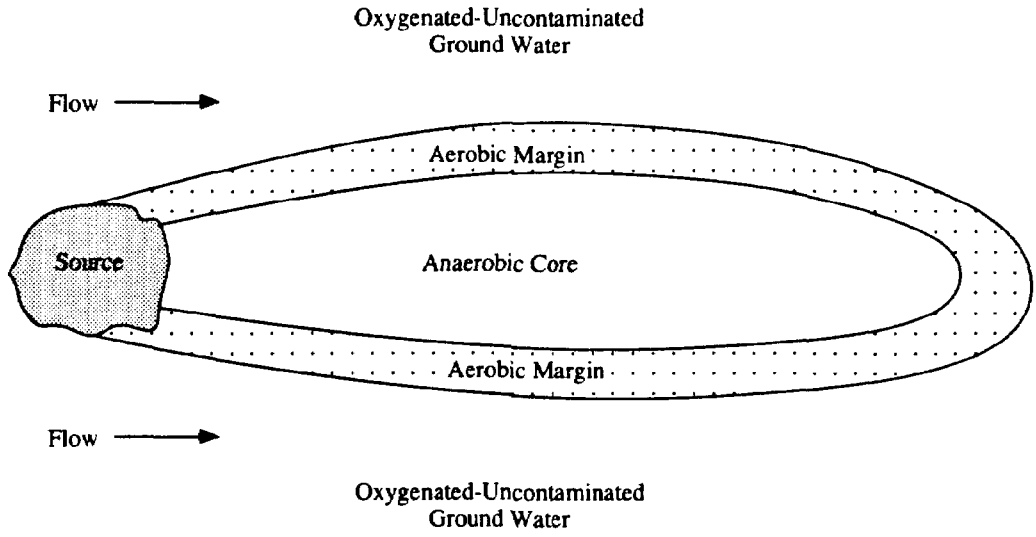

Figure 10. Plan view - hydrocarbon plume undergoing natural biodegradation (Borden, in press).

When it works, natural bioremediation is capable of completely containing a dissolved hydrocarbon plume. While there are few well-documented cases where this has occurred, there is a great deal of anecdotal evidence that suggests that natural bioremediation can be effective in containing dissolved hydrocarbon plumes. Typically, greater than 90% of all underground tanks are used to store gasoline and other petroleum fuels. Yet a study by the California Department of Health Services (Hadley and Armstrong, 1991) found that by far the most common ground-water contaminants were chlorinated solvents, not petroleum constituents. These results suggest that the petroleum contaminants are being removed to below detection limits before reaching water supply wells. Some sites have shown that anaerobic bioremediation processes also occur naturally and can significantly reduce contaminant concentration on aquifer solids and in ground water. Benzene, toluene, ethylbenzene, and xylene can be removed anaerobically in methanogenic or sulfate-reducing environments. Highly chlorinated solvents can undergo reductive dechlorination in anaerobic environments, and evidence exists that this is occurring in-situ at several sites.

While there are no truly typical sites, it may be helpful to consider a hypothetical site where a small release of gasoline has occurred from an underground storage tank (**Figure 11**). Rainfall infiltrating through the hydrocarbon contaminated soil will leach some of the more soluble components including benzene, toluene, and xylenes. As the contaminated water migrates downward through the unsaturated zone, a portion of the dissolved hydrocarbon may biodegrade. The extent of biodegradation will be controlled by the size of the spill, the rate of downward movement, and the existence of appropriate environmental conditions. Dissolved hydrocarbons that are not completely degraded in the unsaturated zone will enter the saturated zone and be transported downgradient within the water table where they will be degraded by native microorganisms to an extent limited by available oxygen or other subsurface conditions. The contaminants that are not degraded will move downgradient under anaerobic conditions. As the plume migrates, dispersion will mix the anaerobic water with oxygenated water at the plume fringes. This is the region where most natural aerobic degradation occurs.

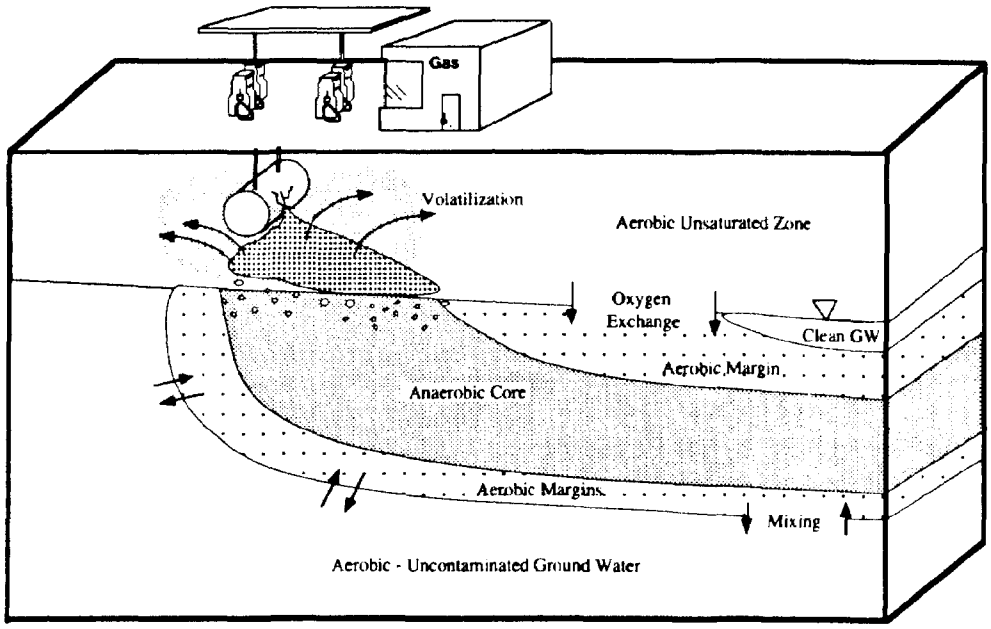

Figure 11. Profile of natural biodegradation of a typical UST release (Borden, in press).

One of the major factors controlling the use of natural bioremediation is the acceptance of this approach by regulators, environmental groups, and the public (Borden, in press). Although natural unaided bioremediation imposes little or no costs other than monitoring and the time for natural processes to proceed, at sites where this approach is strongly opposed the cost may actually be higher than conventional technologies. For example, in some states responsible parties could be allowed to request a reclassification of contaminated ground water to a nonwater supply use. Although in such cases the responsible party would not be required to actively remediate the site, no one has ever filed such a request due to the perception that legal, administrative, and site characterization costs would be excessive and the probability for success would be low. Implementing a natural bioremediation system differs from conventional techniques in that a small portion of the aquifer is allowed to remain contaminated. This results in the necessity of obtaining a variance from existing regulations. Consequently, some type of risk evaluation will usually be required when natural bioremediation is considered.

Another barrier to acceptance is that, currently, there are no reliable methods for predicting the effectiveness of natural bioremediation without first conducting extensive field testing (Borden, in press). This is often the primary reason why this alternative is not seriously considered when evaluating remedial alternatives. Without some reasonable assurances of success, responsible parties are not willing to risk the large sums of money required for legal, administrative, and site characterizations costs.

3.2.2 Case Studies—Natural Bioremediation

One of the earliest studies of natural bioremediation was conducted at the United Creosoting Company site in Conroe, Texas, by a team of researchers from the Robert S. Kerr Environmental Research Laboratory (U.S. EPA) and the National Center for Ground Water Research. Early work (Lee and Ward, 1984; Wilson et al., 1985) demonstrated that an adapted population of creosote degrading

microorganisms was present within the contaminated zone but not in the uncontaminated regions of the aquifer. Later studies correlated creosote biodegradation with the availability of dissolved oxygen (Lee and Ward, 1984). These results were used to develop and calibrate the computer model, BIOPLUME, to simulate hydrocarbon transport and aerobic biodegradation within the aquifer (Borden and Bedient, 1986; Borden et al., 1986). Model results indicated that removal of the contaminant source would be sufficient to contain the hydrocarbon plume and that active remediation by pump and treat would not be required.

Microbiologists from the U.S. Geological Survey have studied two different creosote contaminated aquifers where anaerobic degradation of organic compounds has been observed. Field studies at a contaminated aquifer in St. Louis Park, Minnesota, showed that methane production was occurring in zones within the aquifer that had been contaminated with creosote (Godsy et al., 1983). Later studies demonstrated that the presence of anaerobes (denitrifiers, iron reducers, sulfate reducers and methanogens) was highly correlated with the presence of creosote. More recent work at an abandoned creosote plant in Pensacola, Florida, has shown that a wide variety of organic compounds present in the aquifer were undergoing methanogenic biodegradation and that transport distances in the aquifer could be correlated with biodegradation rates observed in laboratory microcosms (Troutman et al., 1984; Goerlitz et al., 1985).

Monitoring at petroleum contamination sites suggests that biotransformation of petroleum-related compounds under methanogenic conditions may be more common than has generally been assumed. Ehrlich et al. (1985) observed elevated numbers of sulfate-reducing and methanogenic bacteria in a jet fuel contaminated aquifer. Evans and Thompson (1986) and Marrin (1987) monitored methane concentrations in soil gas to map subsurface hydrocarbon contamination. In a study of soil gas concentrations near underground storage tanks, Payne and Durgin (1988) found elevated methane concentrations at over 20% of the 36 sites surveyed. Methane gas production can be so rapid that safety problems occur at some sites. Hayman et al. (1988) had to develop a special apparatus to remove the large quantities of methane generated from a fuel spill at the Miami, Florida, airport.

Hult (1987b) observed the production of large volumes of methane in the unsaturated zone immediately below a crude oil spill at the U.S. Geological Survey research site in Bemidji, Minnesota. At this same site, Eganhouse et al. (1987) observed a two order of magnitude decrease in alkylbenzene concentration over a downgradient travel distance of 150 m. This decrease was accompanied by elevated concentrations of aliphatic and aromatic acids in the ground water (Baedecker et al., 1987). The acids included benzoic, methylbenzoic, trimethylbenzoic, toluic, cyclohexanoic, and dimethylcyclohexanoic. These are the same acids identified by Grbić-Galić and Vogel (1987) as intermediates in anaerobic degradation of alkylbenzenes. Ground-water and sediment analyses demonstrated that methanogenic biodegradation caused a drop in pH and a rise in bicarbonate concentrations in the ground water. The actual drop in ground-water pH appears to have been limited by dissolution of carbonate minerals (and possibly aluminosilicates) (Siegel, 1987).

3.3 Bioremediation: Enhancing Natural Biodegradation

The primary factors limiting natural biodegradation in the subsurface are most often a lack of essential nutrients (i.e., nitrogen, phosphorous), appropriate electron acceptors (oxygen, nitrate, others), and appropriate environmental conditions (pH, redox potential). Most approaches to engineered bioremediation involve different ways to deliver these materials to the contaminated subsurface, where existing microbial populations can then utilize them while degrading contaminants. For example; **in-situ bioremediation** refers to treatment of soils or aquifer materials that are left in place. Air sparging, bioventing, and ground-water recirculation with addition of nutrients and oxygen or H_2O_2 are all

examples of in-situ bioremediation. The major differences are in the approach taken to deliver electron acceptors, and the subsurface location targeted for that delivery. **Air sparging** injects air into contaminated aquifers just below the water table. **Bioventing** utilizes soil vacuum extraction techniques to sweep air through contaminated soils above the water table. **Ground-water recirculation** targets the saturated zone, and uses water as the carrier for nutrients and electron acceptors. In **land treatment**, nutrients can be mixed with soils during tilling or added with irrigation water and oxygen is introduced during the tilling process.

Considerable interest exists in the use of **introduced microorganisms** in bioremediation. Microbes used in such "bioaugmentation" may come from a variety of sources; these include isolation of specific contaminant degraders from sites, laboratory selection of strains with superior degrading capabilities, or production of genetically engineered microbes (**GEMs**). Use of these **exogenous**, or outside, organisms to inoculate the subsurface is generally not considered to be necessary or successful for most in situ bioremediation efforts. However, in situations such as bioreactors or fresh spills of contaminants, distinct applications for use of bioaugmentation; and many advances are being made in biotechnology for degradation of industrial wastes under those circumstances. Use of GEMs is currently controversial, and releases of genetically engineered strains to the environment are strictly regulated. The following section provides information on nutrient, electron acceptors, introduced microorganisms, and other amendments (cometabolic substrates) which are used for enhanced bioremediation.

3.3.1 Nutrients

While a variety of other minerals such as iron, magnesium, and sulfur are required by microorganisms, the primary elements which are necessary for microbial growth include carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and phosphorous (P). The basic premise of contaminant biodegradation is that microbes obtain carbon and energy from organic contaminants and, in the process, convert or transform those materials to simpler compounds. The carbon and energy taken from contaminants are used during growth, along with other elements, to make new cell components. Adequate amounts of nitrogen, phosphorous, and electrons are necessary for microbial metabolism and growth to effectively degrade contaminants. These nutrients must be available to the microbes in: (1) a useable form; (2) appropriate concentrations; and (3) proper ratios (Dragun, J., 1988). Nitrogen and phosphorous are generally not present to a great degree in most contaminants, especially petroleum hydrocarbons primarily composed of carbon and hydrogen. In addition, these elements, although present, may not be plentiful in subsurface soils and aquifers. The other minerals are needed in trace amounts, and adequate amounts are normally found in most ground waters and subsurface materials. On the other hand, there is a considerable surplus of carbon in contaminated subsurface environments. Therefore, biodegradation of contaminants only proceeds until available N and P supplies are depleted.

Several approaches have been used to supply nutrients. Commercial agricultural fertilizers have been applied in solid form or mixed with irrigation water. Types of fertilizers which have been successful include urea-phosphate, N-P-K mixtures, and ammonium and phosphate salts (Atlas, 1984). Raymond et al. (1975, 1978) mixed a blend of approximately equal amounts of ammonium chloride and sodium orthophosphate with ground water which was injected into the contaminated zone of an aquifer. Some practitioners have begun using potassium salts to reduce swelling potential in clays and to tripolyphosphates which will solubilize rather than precipitate iron, calcium, and magnesium (Brown and Norris, 1988). Oleophilic fertilizers, which dissolve into petroleum hydrocarbons, have also been used to stimulate biodegradation of crude oil on beaches in Prince William Sound following the Exxon Valdez oil spill (Pritchard and Costa, 1991).

A variety of C:N:P ratios have been used for bioremediation applications. Atlas and Bartha (1973) reported optimum biodegradation rates of oil in seawater using an oleophilic fertilizer with a C:N:P ratio of 100:10:1. For aerobic treatment, optimal concentrations of ammonia or nitrate nitrogen are in the range of 2 to 8 pounds per 100 pounds of organic material, while inorganic phosphorus requirements are about one-fifth of this (McCarty, 1988). C:N:P ratios of 120:10:1 have been suggested for bioremediation of contaminated soils (USEPA,1989).

These ratios and others found in the literature should only be used as general guidelines. Specific nutrient requirements are not easily predicted since a number of factors other than amount of contaminant may affect the amount of nutrients needed. Sorption, precipitation, and ion exchange of nutrients by geologic materials can substantially increase the amount of nutrients that have to be introduced in order to distribute nutrients across the contaminated zone. Adsorption may be modest in clean sands but may consume most of the nutrients in silts and clays, especially if the solids have a high natural organic content. If all of the hydrocarbon mass were converted to cell material, the nutrient requirements based on the mass of hydrocarbon to be consumed would be approximated by a ratio of carbon to nitrogen to phosphorous of 100:10:1. However, the literature reports significant variations in this ratio, depending on site-specific conditions such as type of contaminant, soil type, microbial population structure, and which metabolic pathway the microbes use to biodegrade the contaminant. For instance, if not all of the hydrocarbon is converted to cell material, but is mineralized to carbon dioxide and water, nutrient requirements may be less than this ratio.

Several bioremediation companies market proprietary nutrient mixtures as part of their services. However, these are not necessary in order to provide nitrogen and phosphorous. In fact, care should be exercised in application of nutrient mixtures with "unknown" ingredients which may have unexpected interactions in the subsurface. Undesired effects include swelling of clays and precipitation of calcium, iron, and magnesium which are all important nutrients.

Two approaches have been used to select appropriate nutrient application rates: (1) addition of nutrients based on the amount of organic carbon present to achieve arbitrarily selected C:N:P ratios and, (2) experimental determination of most effective nutrient mixtures during site-specific treatability studies. The first approach relies on experience obtained under varying conditions and information from published accounts. Although this sort of information is an adequate starting point, the second approach generally avoids surprises caused by site-specific variations in nutrient demands.

3.3.2 Terminal Electron Acceptors

Biodegradation is essentially a series of oxidation-reduction reactions where the contaminant is oxidized (donates electrons) or reduced (accepts electrons). In the process, the contaminant is transformed to simpler molecules and the amount of potential energy in the contaminant is decreased (**Figure 12**). A variety of compounds act as electron acceptors in metabolic pathways. The most critical of these to bioremediation processes are the terminal electron acceptors (TEA), which are usually obtained from the environment outside the cell.

Microbes in nature utilize a variety of compounds as terminal electron acceptors; including oxygen (O_2), nitrate (NO_3^-), iron and manganese oxides (e.g., $Fe(OH)_3$, MnO_2), sulfate ($SO_4^=$), and carbon dioxide (CO_2). Which particular compound is used depends on redox conditions and the type of bacteria present. Aerobic bacteria can only use molecular oxygen (O_2), while anaerobic bacteria can use other compounds such as NO_3^-, $SO_4^=$, $Fe(OH)_3$, or CO_2. Some contaminants are only transformed under

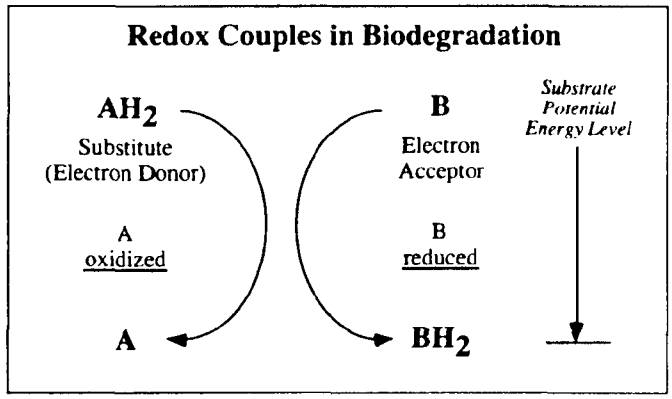

Figure 12. Redox couples in biodegradation.

aerobic conditions, while others require strongly reducing anaerobic conditions, and still others are transformed in both aerobic and anaerobic environments.

Microorganisms preferentially utilize electron acceptors that provide the maximum free energy during respiration. In aquifers contaminated with biodegradable organic compounds, electron acceptors tend to be used successively in order of decreasing oxidation-reduction (redox) potential and free energy yield. Oxygen is the most preferred electron acceptor because it has the highest redox potential and provides the most free energy to microorganisms during electron transfer (**Figure 13**). The redox potentials of nitrate, Mn(IV) and Fe(III) oxides (MnO_2 and FeOOH, respectively), sulfate, and carbon dioxide are lower. As a result, they yield less energy during substrate oxidation and electron transfer according to the order listed in Figure 13. This sequence applies to pH 7 and should be valid for most field conditions where the appropriate microorganisms occur.

The importance of microbial reactions involving Mn(IV) and Fe(III) to organic contaminant biotransformations is unknown. Sulfate and carbon dioxide are the least preferred because microorganisms gain the least energy from these reactions. However, these latter compounds comprise the alternate electron acceptors available for development of anaerobic bioremediation technologies.

3.3.2.1 Oxygen, Hydrogen Peroxide

The most common bioremediation approach is based on aerobic processes. As currently practiced, conventional in-situ biorestoration of petroleum-contaminated soils, aquifer solids, and ground water relies on the supply of oxygen to the subsurface to enhance natural aerobic processes to remediate the contaminants. It has been recognized that the rate at which oxygen can be introduced by sparging air in a ground-water injection well limits the effectiveness of the technology. Since the amount of oxygen which can be added to water from air is limited (8-10 mg/l), other sources of oxygen have been used. These include pure oxygen and hydrogen peroxide. Use of pure oxygen in place of air can increase the rate of introduction of oxygen five-fold.

Hydrogen peroxide is commonly used as a method of introducing oxygen (Brown et al., 1984). This liquid, which decomposes to oxygen and water, is completely soluble in water. Because of this using hydrogen peroxide can, theoretically, provide oxygen 5-50 times faster than could sparging air or pure oxygen into injection wells and should result in shorter remediation times. However, the efficiency of delivering oxygen by this method has been quite variable even when favorable results were obtained

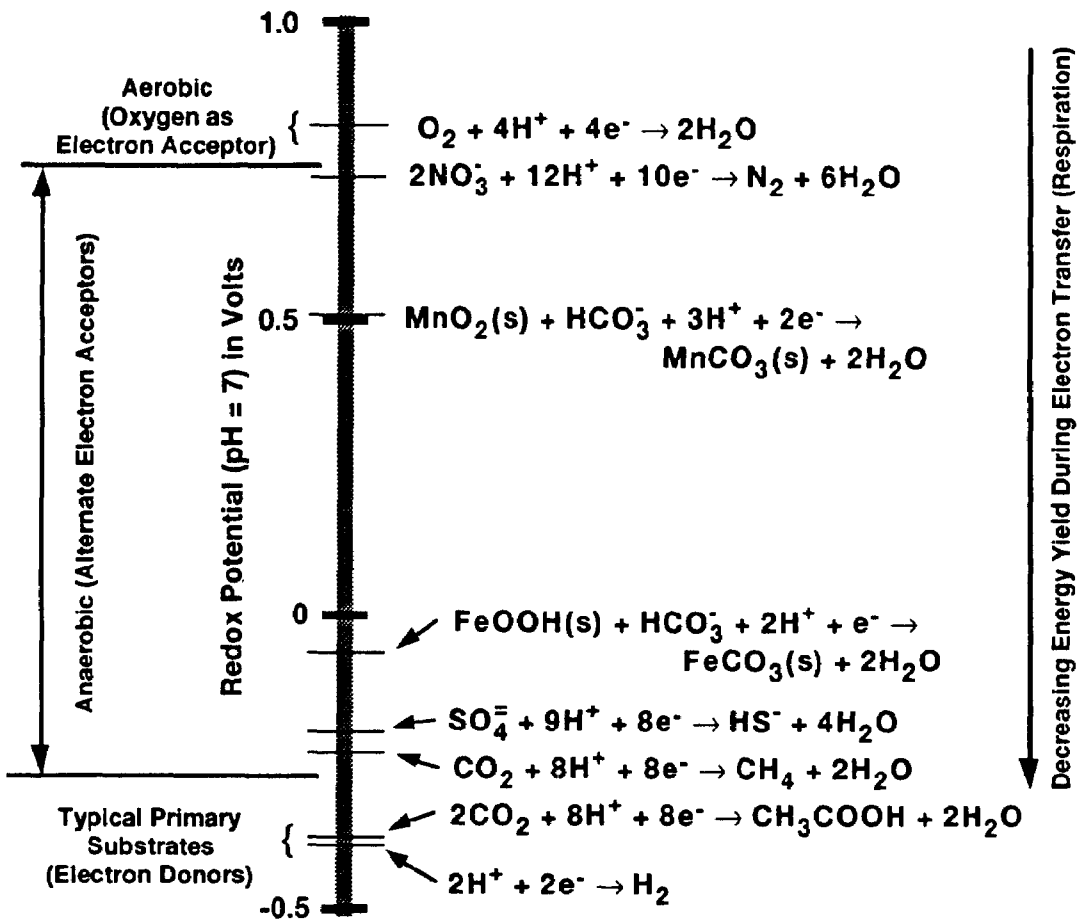

Figure 13. **Redox potential and energy yields of electron acceptors and donors important in bioremediation (Bouwer, in press).**

from laboratory screening tests (Lawes, 1991; Huling et al., 1990; Hinchee and Downey, 1988; and Flathman et al., 1991). Hydrogen peroxide breaks down rapidly in contact with soils and organic contaminants. In aquifers, the released oxygen may migrate rapidly out of the saturated zone, resulting in poor delivery of oxygen to contaminated zones. Further, as microbial populations decrease as a function of decreasing food source (the contaminants), tolerance toward hydrogen peroxide may also decrease. Practical considerations, including toxicity towards bacteria and precipitation of iron and phosphate, limit hydrogen peroxide concentrations to 100 to 1,000 ppm. In addition, too much oxygen may stimulate biofouling of well screens and aquifers. As a result, hydrogen peroxide may not be the most appropriate oxygen source for many sites.

3.3.2.2 Alternate Electron Acceptors—Nitrate, Sulfate, Carbon Dioxide

Rapid aerobic degradation requires an ample supply of nutrients and oxygen, good mixing, and a high microbial mass. These conditions are often difficult to maintain in aquifers (Wilson, B. et al., 1986; Lee et al., 1988). Water is a poor mass transfer medium for O_2 due to the low water solubility of O_2.

Because of this, degradation of relatively small amounts of hydrocarbons requires that large amounts of water come in contact with the aquifer solids. The complete oxidation of 1 mg of hydrocarbon compounds requires 3.1 mg of O_2 (Hutchins and Wilson, 1991). Thus, for the bioremediation of 1 kg of aquifer material containing 10 g/kg hydrocarbon compounds, a minimum of 3.1 m^3 of oxygenated water containing 10 mg/l O_2 must be supplied. Furthermore, at many sites there may be a very high abiotic oxygen demand due to hydrogen sulfide (H_2S), iron (Fe^{2+}), or other readily oxidizable compounds. This may make it difficult to increase the reduction potential into the aerobic range (> + 0.82 volts). Therefore, anaerobic conditions are expected to persist within aerobically treated aquifers, especially in relatively impermeable zones and zones farther away from the injection wells.

Bioremediation using electron acceptors other than oxygen is potentially advantageous for overcoming this difficulty in supplying oxygen for aerobic processes. When oxygen is consumed faster than it can be supplied, anaerobic microorganisms may grow using alternate electron acceptors. Nitrate, sulfate, and carbon dioxide are attractive alternatives to oxygen because they are more soluble in water, inexpensive, and nontoxic to microorganisms. Anaerobic microbial processes can be significant in oxygen-depleted subsurface environments that are contaminated with petroleum-based compounds and/or chlorinated solvents. The dark shaded area in **Figure 14** illustrates the location of contaminants that may be remediated by introduction of alternate electron acceptors. In the absence of molecular oxygen, microbial reduction reactions involving organic contaminants increase in significance as environmental conditions become more reducing. In this environment, some contaminants are reduced by a biological process known as reductive dehalogenation. In reductive dehalogenation reactions, the halogenated compound becomes the electron acceptor. In this process, a halogen is removed and is replaced with a hydrogen atom. Reductive dehalogenation is a significant anaerobic degradation process which has been shown to work on such recalcitrant compounds as polychlorinated biphenyls (PCBs), organochlorine pesticides (DDT, toxaphene), and chlorinated solvents (PCE,TCE).

Anaerobic degradation of aromatic hydrocarbons was initially identified at field sites (Reinhard et al., 1984) and in microcosm studies (Wilson et al., 1987) and has now been demonstrated in the

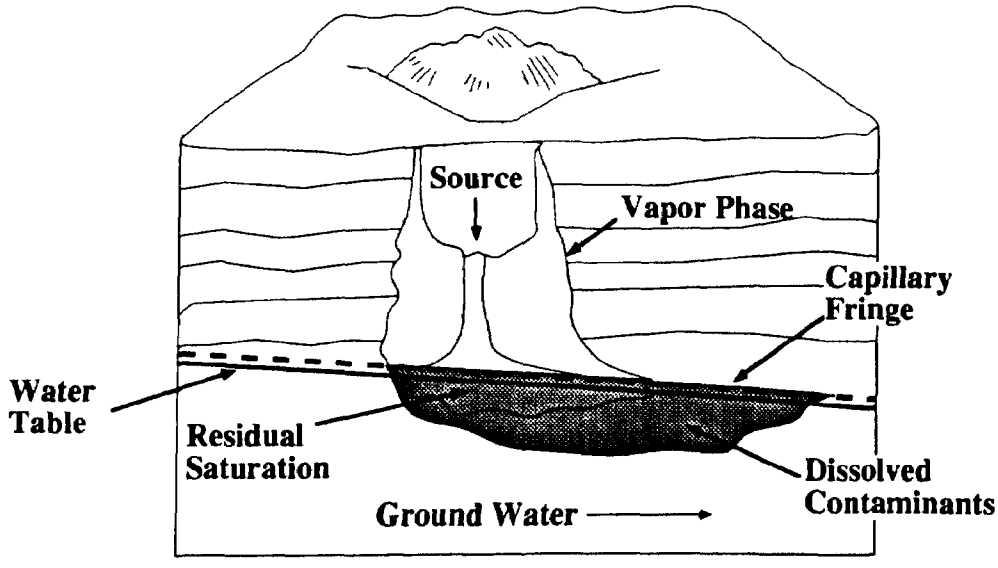

Figure 14. Contaminant locations treatable with alternate electron acceptors.

laboratory using a number of redox conditions and electron acceptors, including reduction of nitrate, iron(III) and manganese(IV) oxides, sulfate, and carbon dioxide. In contrast to aromatic hydrocarbons, aliphatic hydrocarbon degradation without oxygen has not been reported. The feasibility of alternate electron acceptors other than nitrate for bioremediation has not been documented at field scale but has been widely studied at laboratory scale. The other possible alternate electron acceptors (i.e., iron(III), sulfate, and CO_2) have been found in systems that may be classified as passive bioremediation, such as in landfill leachate plumes (Reinhard et al., 1984) and at spill sites (Lovley et al., 1989). Nitrate has been used as an electron acceptor for bioremediation of benzene, toluene, ethylbenzene, and xylenes in ground water and on aquifer solids.

3.3.2.2.1 Nitrate and Denitrifying Systems

Microbial populations capable of using nitrate as an electron acceptor are widespread in the environment. **Denitrification** results when bacteria utilize nitrate and convert it to N_2, which then leaves the subsurface system as a gas. Nitrate salts are much more water soluble (92 g/l as sodium nitrate) than O_2 (10 mg/l). Approximately 50 times more reducing equivalent can be introduced into an aquifer using saturated sodium nitrate solution rather than a saturated oxygen solution. Under denitrifying conditions, oxidation of monoaromatic compounds has been demonstrated in a number of systems (e.g., Kuhn et al., 1988; Mihelcic and Luthy, 1988; Altenschmidt and Fuchs, 1991; Ball et al., 1991; Evans et al., 1991a; Evans et al., 1991b; Flyvbjerg et al., 1991; Hutchins et al., 1991a; Evans et al., 1992).

When biodegradation of benzene, toluene, ethylbenzene and xylenes (BTEX) mixtures was tested under denitrifying conditions, degradation tended to be sequential, with toluene being the first substrate to be degraded, followed by *p*- and *m*-xylene, ethylbenzene and *o*-xylene. Benzene does not seem to be degraded (Kuhn et al., 1988; Evans et al., 1991a; Evans et al., 1991b; Hutchins et al., 1991a) although in one study Major et al. (1988) reported removal under conditions thought to be denitrifying. Hutchins et al. (1991a) reported longer lag times and slower degradation rates in core material contaminated with JP-4 aviation fuel than in uncontaminated core material. Using an enrichment culture and ethylbenzene as the substrate, Ball et al. (1991) have shown that single aromatic substrates can be degraded rapidly (within hours) and that nitrate reduction to nitrogen gas proceeds through nitrite. Similar findings were reported by Evans et al. (1991a, 1991b) for toluene. Ball et al. (1991) also demonstrated that composition and preparation of the growth medium can affect the observed transformation rates. Degradation of PAHs (naphthalene) under denitrifying conditions has also been reported (Mihelcic and Luthy, 1988) .

3.3.2.2.2 Iron(III) Reducing Systems

Once available oxygen and nitrate are depleted, subsurface microorganisms may use oxidized ferric iron [Fe(III)] as an electron acceptor. Microorganisms have been identified that can couple the reduction of ferric iron with the oxidation of aromatic compounds including toluene, phenol, *p*-cresol and benzoate (Lovley and Lonergan, 1990; Lovley et al., 1989). Large amounts of ferric iron are present in the sediments of most aquifers and could potentially provide a large reservoir of electron acceptor for hydrocarbon biodegradation. This iron may be present in both crystalline and amorphous forms. The forms that are most easily reduced are amorphous and poorly crystalline Fe(III) hydroxides, Fe(III) oxyhydroxides, and Fe(III) oxides (Lovley, 1991).

Lovley and Lonergan (1990) have isolated an iron-reducing bacterium capable of degrading toluene, *p*-cresol and phenol. Relative to other anaerobic processes, Fe(III) reduction has a very unfavorable substrate to electron acceptor ratio. Transport of the dissolved iron Fe(II) from the aquifer

could cause secondary problems such as clogging and fouling of the aquifer. Furthermore, the supply of large amounts of colloidal iron(III)oxide or soluble Fe(III)citrate (Lovley et al., 1989) to an aquifer has not been tested. To develop bioremediation strategies based on iron reduction, a better understanding of occurrence, nutritional requirements, growth conditions and metabolism of iron-reducing bacteria must be developed.

3.3.2.2.3 Sulfate and Sulfate Reducing Systems

Bioremediation using sulfate as the electron acceptor involves oxidation of aromatic hydrocarbons by sulfidogenic organisms coupled with reduction of sulfate to hydrogen sulfide (Edwards et al., 1991; Haag et al., 1991; Beller et al., 1992; Edwards et al., 1992). As in some denitrifying systems, degradation under sulfate reducing conditions is also sequential; with toluene being the preferred substrate, followed by *p*-xylene and with *o*-xylene degraded last (Edwards et al., 1991, 1992). Ethylbenzene and benzene were not degraded under the conditions of the experiment. In a follow-up study, Edwards and Grbić-Galić (1992) observed benzene degradation in the absence of all other aromatic substrates. After a lag time of 30 days under strictly anaerobic conditions, these authors observed mineralization of benzene and suspected sulfate to be the electron acceptor. Accumulation of HS^- may inhibit the process, however, and is a problem that remains to be resolved.

3.3.2.2.4 Fermentative/Carbon Dioxide Reducing Systems

Under very reduced conditions, anaerobic organisms utilize carbon dioxide as an electron acceptor and produce methane (CH_4). These conditions are therefore known as **methanogenic.** Under methanogenic/fermentative conditions, several aromatic hydrocarbon compounds, including benzene and toluene, have been shown to transform into CO_2 and methane (Grbić-Galić and Vogel, 1987). Biotransformation under these conditions was studied with toluene or benzene as the only carbon source. Biotransformation began after a three-month lag time and was complete after 60 days of incubation. Since this ground-breaking study, several other aromatic substrates have been shown to be degraded under methanogenic conditions, including styrene, naphthalene and acenaphthalene (Grbić-Galić, 1990), as well as benzothiophene, a sulfur-containing heterocyclic compound (Godsy and Grbić-Galić, 1989).

Fermentation/methanogenic degradation could be used as a passive bioremediation technology and is likely to be an ongoing process at many sites where the geochemical conditions have evolved naturally, without human intervention. Reliable assessment of the process is difficult under field conditions since mass balances are difficult to establish. Indications for the process are the occurrence of methane in combination with characteristic intermediates such as aromatic acids (Reinhard et al., 1984; Wilson et al., 1987; Baedecker and Cozzarelli, 1991).

3.3.2.2.5 Mixed Electron Acceptor Systems

Few laboratory studies have examined mixed electron acceptor systems, although they are likely to be common at field sites. Terminal electron acceptors such as sulfate and carbon dioxide are likely to co-occur naturally, either within the same aquifer compartment or spatially separated into adjacent compartments. For instance, at the sites where denitrifying conditions were investigated, O_2 was frequently present in the nitrate feed water. Both electron acceptors were consumed, but the effect of the oxygen on the overall process was not determined. Werner (1985) proposed that if O_2 and nitrate are present simultaneously, O_2 is used for the first oxidation step to produce partially oxygenated products and nitrate is then used for mineralization of the oxidation products.

Different electron acceptors and products of aromatic degradation processes can react with each other in a number of biological and chemical reactions. Beller et al. (1992) have studied the link between sulfate reduction to sulfide and iron(III) reduction to iron(II) by a sulfate-reducing enrichment culture. Ferric iron appeared to reoxidize hydrogen sulfide in an abiotic process and/or lower the inhibitory effect of hydrogen sulfide. Toluene was the sole carbon and energy source, but other substrates were not tested.

Anaerobic bioremediation has been tested only in a very few cases and is still considered experimental. For instance, in a review of 17 sites contaminated with hydrocarbon fuels and oils (Staps, 1990), hydrogen peroxide was used as the electron acceptor at seven sites, air at five, combinations of nitrate-ozone and nitrate-air at one site each, and nitrate alone was used only at three sites. Much available information has been developed in laboratory studies; however, the applicability of these results to field conditions remains to be studied. Anaerobic transformation rates can be slow and lag times long and unpredictable, except for transformation in denitrifying systems which can be fast. In spite of slow rates, anaerobic bioremediation could play a significant role in the future mainly because the principal factor limiting aerobic bioremediation, the difficulty of supplying oxygen to the subsurface, is circumvented.

The combination of an anaerobic process followed by an aerobic process has promise for the bioremediation of highly chlorinated organic contaminants. Generally, anaerobic microorganisms reduce the number of chlorines on a chlorinated compound via reductive dechlorination. Aerobic microorganisms are more capable of transforming compounds with fewer chlorinated substitutes. With the removal of chlorines, oxidation becomes more favorable than does reductive dechlorination. Therefore, the combination of anaerobic and aerobic processes has potential as a control technology for chlorinated solvent contamination.

3.3.3 Introduced Microorganisms

Inocula of microorganisms have been widely used for bioremediation of hazardous waste sites. However, there is little documentation of the efficacy of this process; and important questions still persist about the environmental responsibility of adding nonindigenous, exogenous microorganisms. Microorganisms have been added to samples of soil and water in the laboratory and field to enhance biodegradation of hydrocarbons; however, the results of these studies have been mixed. Atlas (1977) stated in a review on stimulated petroleum biodegradation that seeding will not be necessary in most environments because of the ubiquity of hydrocarbon-degrading organisms. Although hydrocarbon-degrading organisms may be ubiquitous, the problem with natural bioremediation of these compounds is that the rate of biodegradation is often too slow (Thomas and Ward, in press). Nutrient addition and agents that render the compounds more bioavailable may enhance these rates. However, inoculation may be important in environments in which the population of hydrocarbon-degrading organisms is too low or absent, or the environment is too harsh. In the latter case, the added organisms must be able to tolerate the extreme conditions. In addition, inoculation may be beneficial in the biodegradation of the high-molecular-weight polycyclic aromatic hydrocarbons, which are recalcitrant (Bossert and Bartha, 1986). If seeding is considered as a method for hydrocarbon remediation, a mixture of microorganisms will be required. Zajic and Daugulis (1975) found that multiple species were required to degrade the complex composition of crude oil. Ball et al. (1991) tested inocula from different sources for the potential to degrade BTEX compounds. They found that microorganisms with the ability to degrade aromatic hydrocarbons are not ubiquitous. Sewage seed that contains a diverse population of microorganisms, for instance, did not adapt to the aromatic compounds tested.

Operations in which seed organisms are added to enhance contaminant biodegradation in the subsurface usually involve treating contaminated ground water in an aboveground bioreactor, and then reinjecting the treated water into the subsurface. The treated ground water that is reinjected contains adapted microorganisms from the bioreactor or is amended with contaminant-degrading organisms to enhance in-situ biodegradation (Ohneck and Gardner, 1982; Quince and Gardner, 1982a, b; Winegardner and Quince, 1984; Flathman and Githens, 1985; Flathman and Caplan, 1985, 1986; Flathman et al., 1985; Quince et al., 1985).

3.3.3.1 Microbial Transport

Seed microorganisms have been added to the subsurface to aid in contaminant biodegradation; however, the role of the added microorganisms has never been differentiated from that of the indigenous microflora (Lee et al., 1988; Thomas and Ward, 1989). For added organisms to be effective in contaminant degradation, they must be transported to the zone of contamination, attach to the subsurface matrix, survive, grow, and retain their degradative capabilities. There are a number of phenomena which affect the transport of microbes in the subsurface including grain size, cracks and fissures, removal by sorption in sediments high in clay and organic matter, and the hydraulic conductivity. Many other factors affect the movement of microorganisms in the subsurface, including their size and shape, concentration, flow rate, and survivability.

The concept of microbial movement through the subsurface was first addressed as early as the mid 1920s for microbial enhanced oil recovery (MEOR). At that time, Beckmann (1926) suggested that microorganisms that produce emulsifiers or surfactants could be transported into an oil-bearing formation to recover oil that remains after a well has stopped flowing. The addition of microorganisms to oil-bearing formations to enhance oil recovery by biosurfactant or biogas production has since been investigated and appears promising (Bubela, 1978). At about the same time, research on the transport of microorganisms through the subsurface environment was being conducted to determine the effectiveness of on-site wastewater disposal systems (i.e., pit latrines, septic tanks, land disposal of sewage) in removing pathogens (Caldwell, 1937, 1938). More recently, the concept of transporting microorganisms with specialized metabolic capabilities for subsurface bioremediation has been proposed (Lee et al., 1988; Thomas and Ward, 1989) In recent years, research has been directed toward the introduction of microorganisms to soil and ground water to introduce specialized metabolic capabilities, to degrade contaminants which resist the degradative processes of indigenous microflora, or when the subsurface has been sterilized by contaminants. One of the first studies that addressed microbial transport through subsurface materials for the purpose of contaminant degradation was published by Raymond et al. (1977). These investigators reported that heterotrophs and hydrocarbon-degrading bacteria penetrated and were detected in the effluent of 1.45 x 31 cm columns packed with unconsolidated sands having effective hydraulic conductivity (K) values ranging from 3.38×10^{-3} to 1.9×10^{-1} cm/sec, which were run at a flow rate of about 30 ml/h (Darcy flow 18/ cm/hr). Microorganisms also penetrated and were detected in the effluent of 3.8 x 10 cm sandstone (consolidated) cores, with hydraulic conductivities ranging from 1.8×10^{-5} to 7.2×10^{-5} cm/sec, through which water was passed under unknown pressure. In a separate experiment, it was determined that the added microorganisms were utilizing the gasoline.

3.3.3.2 Factors Affecting Bacterial Transport and Survival in the Subsurface

3.3.3.2.1 Matrix

Matrix properties that affect microbial transport in the subsurface include hydraulic conductivity, mineralogy, and sediment structure. Hydraulic conductivity has been the most studied

parameter affecting transport through porous media; however, the results of laboratory studies in which samples of porous media were packed to homogeneity may produce underestimates of microbial transport. The use of intact cores will provide the full range of pore sizes present in situ for microbial transport through available macropores. Marlow et al. (1991) reported that extent of transport of a yeast, *Rhodotorula* sp., after 10 pore volumes through sand columns with hydraulic conductivity values of 5.59 x 10^{-2} and 1.37 x 10^{-1} cm/sec was about 2 and 50%, respectively, of the initial number of cells added (1 to 2 x 10^{5} cells/ml). Fontes et al. (1991) investigated the effects of grain size, bacterial cell size, ionic strength of the transporting fluid, and heterogeneities of the medium on microbial transport and found that grain size was the most important variable.

3.3.3.2.2 Properties of Microbes

The properties of the microorganisms that may affect transport include size, aggregating tendencies, shape, condition, and motility and chemotaxis. The results of studies designed to investigate which cell characteristics are most important are mixed. Cell size is important in that transport will be limited or prevented for cells that are bigger than the average pore size; however, cells that tend to aggregate, even if they are small, will not be good candidates for transport. For microorganisms that form spores, the spore, which is smaller than the vegetative stage, may be transported more efficiently. Microorganisms that are in a starved state usually are smaller, and produce less extracellular polysaccharide, which allows the organism to attach to surfaces. Thus the reduced size and stickiness of the cells should enhance transport.

3.3.3.2.3 Operational Factors

The operational factors that will affect microbial transport include cell concentration, flow rate, and the ionic strength of the transporting fluid. The results of studies designed to investigate the effects of cell density on transport have been mixed. The effects of cell density on transport may be organism- and site-specific. Microbial filtration and clogging of the matrix will be of concern. A direct relationship exists between flow rate and microbial transport. Finally, there is an inverse relationship between the ionic strength of the transporting fluid and microbial transport. Microorganisms tend to sorb to surfaces under conditions of high ionic strength. Therefore, more cells will be transported in a fluid of low ionic strength. The results from a number of studies suggest that in-situ bioremediation of the subsurface is usually limited to formations with hydraulic conductivities of 10^{-4} cm/sec (100 ft/yr) or greater to overcome the difficulty of pumping fluids through contaminated formations.

3.3.3.2.4 Survival in the Subsurface

Little information is available concerning the survivability of introduced microorganisms in the subsurface (Thomas and Ward, in press). Transported organisms must not only reach the zone of contamination but must compete with the indigenous microflora for nutrients, escape predation, retain their biodegradative capabilities, and often tolerate extremes in pH, temperature, and other environmental variables (Thomas and Ward, in press). Hardly anything is known about environmental factors and survivability in the subsurface environment. The same factors that affect survival of microorganisms in the surface soil and water environments will affect the survivability in the subsurface. These factors include substrate concentrations, pH, temperature, and the presence of toxicants, predators, and alternate substrates. By extrapolation from experience with surface water and soil, predation will probably be the most important factor limiting the survival and activity of introduced microorganisms.

Although specialized microorganisms that have been cultured using selective enrichment techniques can be used in environmental applications, those developed using genetic engineering

techniques cannot be released into the environment for commercial purposes without prior government approval (Pimentel et al., 1989). Genetically engineered microorganisms for use in such operations as MEOR, bioremediation of Superfund sites, extraction and concentration of metals, and production of specialty chemicals, may be regulated under the Environmental Protection Agency's Toxic Substances Control Act, Section 5 (Clark, 1992). The use of microorganisms with specialized capabilities to enhance bioremediation in the subsurface is an undemonstrated technique. However, research has been conducted to determine the potential for microbial transport through subsurface materials, public health effects, and microbial enhanced oil recovery. The use of introduced microorganisms has proven most successful in surface bioreactors when treating ground water in closed-loop systems.

Since the study published by Raymond et al. (1977), which indicated that microorganisms can be transported and enhance degradation of hydrocarbons in a column packed with sand, no one has conclusively demonstrated that inoculation of the subsurface enhances bioremediation in the laboratory or field (Thomas and Ward, in press). However, a significant amount of research tends toward working with organisms that are easy to culture, and whose genetics are well understood. Little consideration has been given to developing organisms with good transport properties and survival traits. These include ability to survive in the subsurface environment, escape predation, and biodegrade the contaminants under in-situ conditions. The best opportunities involve development of inocula that can degrade mixed wastes, that have increased tolerance to toxicants, and that produce bioemulsifiers and biosurfactants to increase their access to oily phase contaminants.

3.3.4 Cometabolic Substrates

Organic compounds can be biotransformed by microorganisms through two basically different processes: (1) use as a primary substrate, and (2) cometabolism (McCarty and Semprini, in press). In the first process, biodegradation occurs when the organism consumes an organic compound as a primary substrate to satisfy its energy and organic carbon needs. Cometabolism, on the other hand, is the fortuitous transformation of an organic compound by enzymes or cofactors produced by organisms for other purposes. Here, the organisms obtain no obvious or direct benefit from the transformation. Indeed, it may be harmful to them.

Cometabolism may occur under either aerobic or anaerobic conditions. For cometabolism to occur, an active population of microorganisms having the cometabolizing enzymes or cofactors must be present. Cometabolism requires that an appropriate primary substrate for growth and maintenance of these organisms must also be present. The primary substrate must often be added to the aquifer, along with an electron acceptor such as oxygen or nitrate for its oxidation. This is an aspect that adds greater complexity and cost to cometabolic bioremediation. Cometabolic substrates which have been investigated include methane, propane, acetate, toluene, and relatively low molecular weight PAHs such as naphthalene. Contaminants which have been shown to be degraded through cometabolic processes include chlorinated aliphatic solvents, high molecular weight polyaromatic hydrocarbons, PCBs, and some pesticides. However, cometabolism has not been used extensively for treatment of organic wastes; knowledge of practical application of these processes and factors affecting performance is quite limited.

SECTION 4
TYPES OF IN SITU BIOREMEDIATION SYSTEMS

Delivery of Nutrients and Electron Acceptors

As noted earlier, bioremediation technologies differ primarily in the method used to deliver nutrients, electron acceptors, and other amendments to contaminants in the subsurface. Development of innovative delivery systems has been the primary focus of research and still remains as the major challenge in successful application of in-situ bioremediation.

Both subsurface environment (geology and hydrology) and nature and distribution of contaminants ultimately determine which delivery approach is most appropriate. Accurate information concerning contaminant nature and distribution, site hydrogeology and geochemistry, and predominant microbial populations is a vital prerequisite for selection and design of bioremediation technologies.

The specific electron acceptors and nutrients which are selected for delivery depend on the type of microbial metabolism to be stimulated. The amounts of these materials to be delivered should be determined with well-designed treatability studies coupled with adequate site characterization.

4.1 In-situ Bioremediation of Soil and Ground Water

In-situ bioremediation technology for the decontamination of soil and ground water contaminated with petroleum-derived hydrocarbons involves the stimulation of naturally occurring microorganisms that are capable of degrading the organic contaminants (Atlas, 1981; Lee et al., 1988). In-situ bioremediation systems for aquifers typically consist of extraction points, such as wells or trenches, and injection wells or infiltration galleries (**Figure 15**).

Contaminant locations most often treated with in-situ bioremediation are shown by the dark shaded area in **Figure 16**. In most cases, extracted ground water is treated prior to the addition of oxygen and nutrients, followed by subsequent reinjection. Ground-water treatment has frequently consisted of an air-stripper tower or activated carbon, but may incorporate an oil/water separator, a biological treatment unit, an advanced oxidation unit, or combinations of treatment units. When recovered ground water contains more than a few ppm of biodegradable substances, treatment is likely to be required by regulations, and is efficient from a process economics perspective. When the recovered ground water contains low levels of readily degradable constituents, they will generally be degraded within a short distance of the injection point and will not add significantly to oxygen and nutrient requirements.

Ground-water flow rate and **flow paths** are critical to the design of in-situ bioremediation systems. The ground-water flow rate must be sufficient to deliver the required nutrients and oxygen according to the demand of the organisms. Amended ground water should sweep the entire area requiring treatment, and the recovery wells should capture the injected ground water to prevent migration outside the designated treatment zone. In order to ensure that adequate control can be maintained over the ground water, usually only a portion of the recovered ground water is reinjected. After appropriate treatment, the other portion is discharged by an acceptable method. The hydraulic conductivity of the ground-water system, or variability of the aquifer materials, often limits the effectiveness of in-situ technologies or entirely prevents their use. A suggested target for in-situ remediation technologies is a hydraulic conductivity of at least 10^{-4} cm/sec (100 ft/yr). The design of a ground-water recirculation system is best

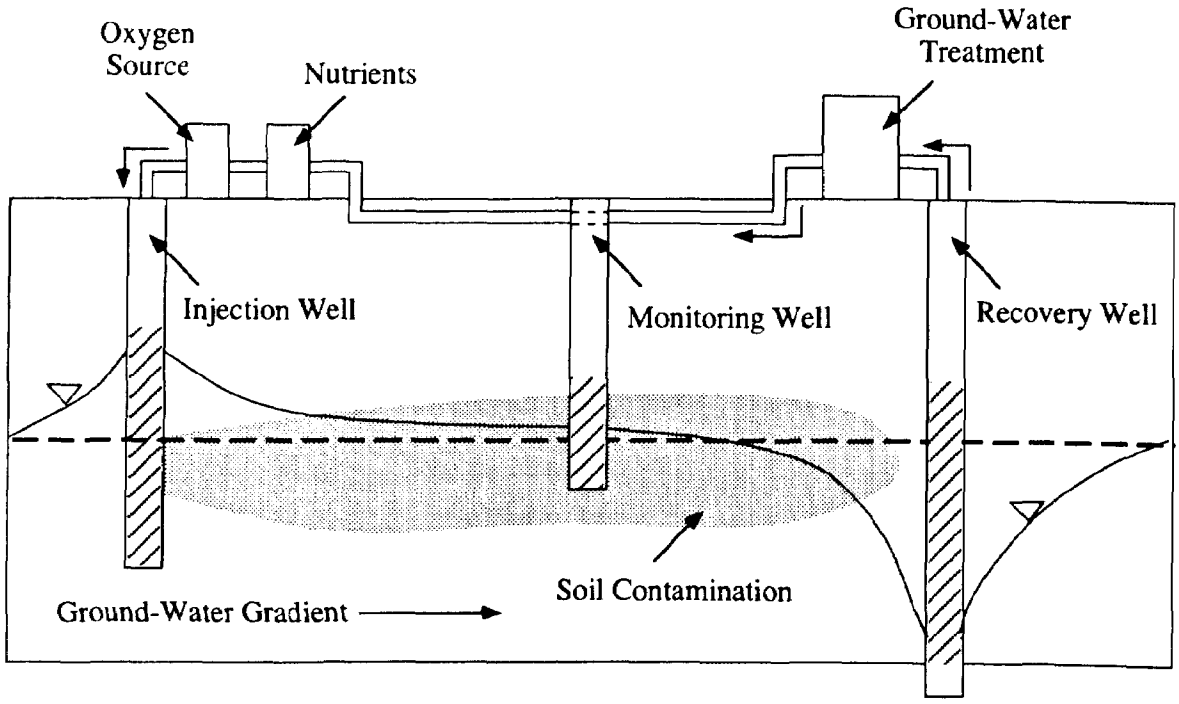

Figure 15. In-situ bioremediation utilizing ground-water extraction and reinjection.

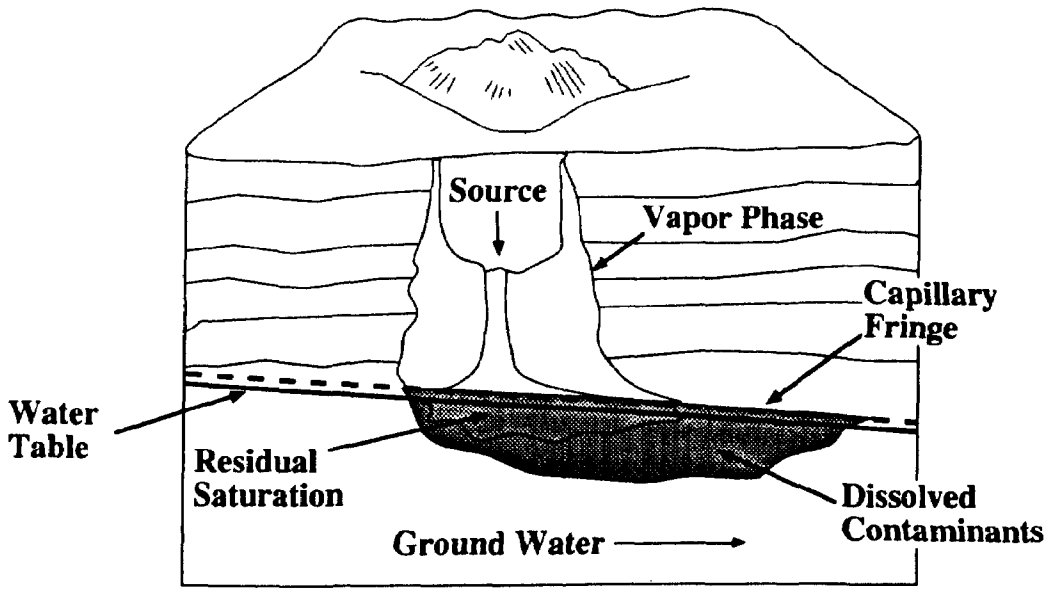

Figure 16. Contaminant locations treated with in-situ bioremediation.

done using a ground-water flow model to evaluate optimum placement of injection/extraction wells (Falatico and Norris, 1990). Such models allow several design concepts to be evaluated and can be more effective than laboratory treatability studies to determine feasibility. In addition, modeling results can be used to make midcourse modifications in operations. For most sites, a two-dimensional analytical flow model will be sufficient. Models which incorporate biodegradation may also be used as an aid in design and to evaluate bioremediation system performance.

In-situ bioremediation systems are often integrated with other remediation technologies, either sequentially or simultaneously. For example, if free-phase hydrocarbons are present, a recovery system should be used to reduce the mass of free-phase product prior to the implementation of bioremediation. In-situ vapor stripping can be used to both physically remove volatile hydrocarbons and to provide oxygen for bioremediation. These systems can also reduce levels of residual phase hydrocarbons as well as constituents adsorbed to both unsaturated soils and soils which become unsaturated during periods when the water table is lowered.

4.2 Air Sparging

Air sparging is the injection of air under pressure directly into a saturated formation below the water table to create a transient air-filled space by displacing water from the soil matrix (**Figure 17**).

Air sparging effectively removes contamination below the water table. Air sparging can successfully treat VOCs and petroleum hydrocarbons in ground-water aquifers through direct volatilization and biodegradation. Air sparging is a remediation technology applicable to contaminated

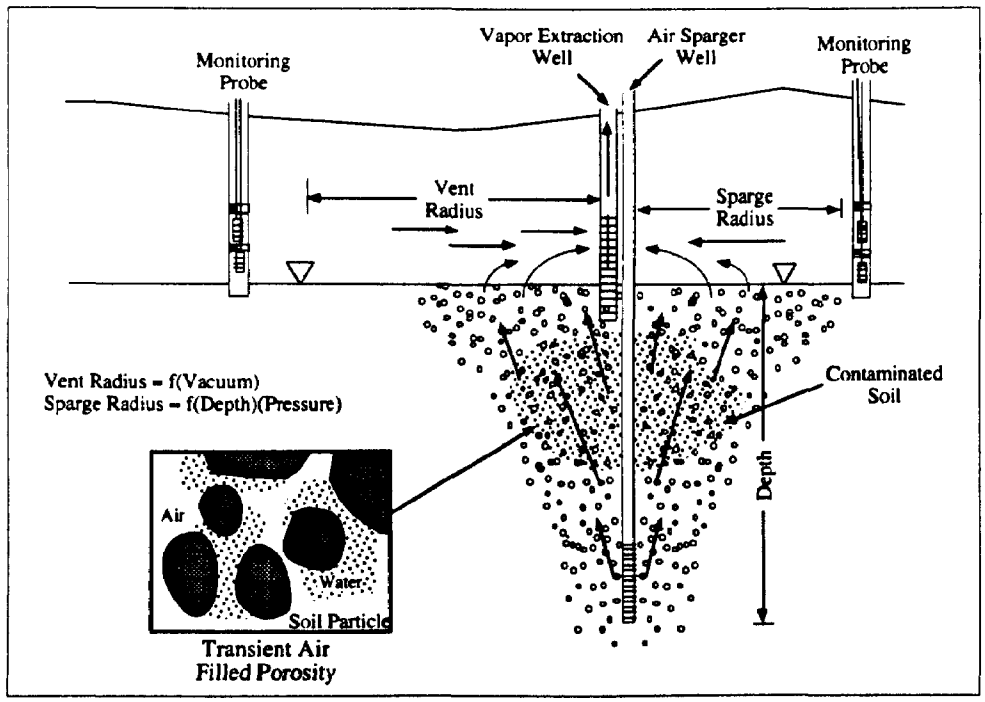

Figure 17. Diagram of air sparging system (Brown, in press).

aquifer solids and vadose zone materials (**Figure 18, dark shaded region**). This is a relatively new treatment technology which enhances biodegradation by increasing oxygen transfer to the ground water while promoting the physical removal of organics by direct volatilization. Air sparging has been used extensively in Germany since 1985 (Hiller and Gudemann, 1988) and was successfully introduced in the United States in 1990 (Brown, et al., 1991; Marley et al., 1990; Middleton and Hiller, 1990).

When air sparging is applied, the result is a complex partitioning of contaminants between the adsorbed, dissolved, and vapor states. Also, a complex series of removal mechanisms are introduced, including the removal of volatiles from the unsaturated zone, biodegradation, and the partitioning and removal of volatiles from the fluid phase. The mechanisms responsible for removal are dependent upon the volatility of the contaminants. With a highly volatile contaminant, for example, the primary partitioning is into the vapor phase; and the primary removal mechanism is through volatilization. By contrast, contaminants of low volatility partition into the adsorbed or dissolved phase; and the primary removal mechanism is through biodegradation. **Figure 19** illustrates the relative importance of volatilization and biodegradation as a function of product volatility.

One of the problems in applying air sparging is controlling contaminant migration. In either bioventing or ground-water extraction, the systems are under control because contaminants are drawn to the point of collection. However, air injected into saturated formations may travel in unpredictable directions and distances. This can mobilize volatile contaminants to the vadose zone or can accelerate migration of dissolved contaminants by creating locally higher ground-water gradients. Vertical barriers to upward migration may trap air and produce lateral spread. VOC-laden air streams can rapidly migrate through the vadose zone to low pressure regions such as basements, causing a vapor hazard. Physical displacement of the water column can occur if too much pressure is used, increasing migration of ground

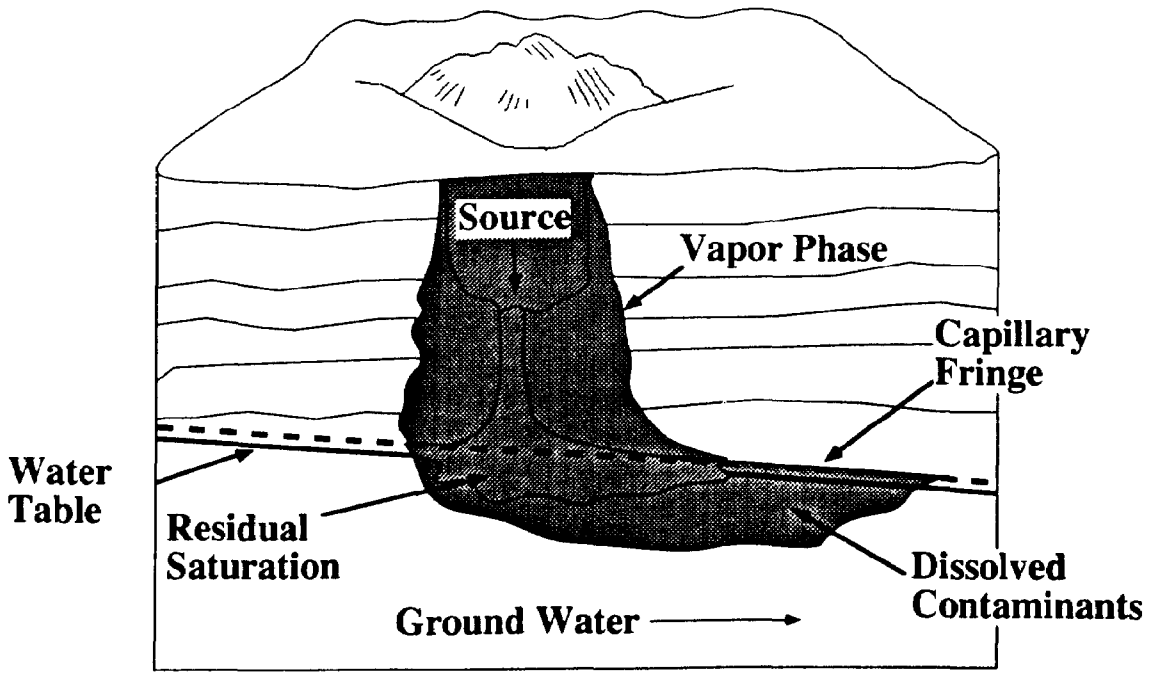

Figure 18. Contaminant locations treatable with air sparging.

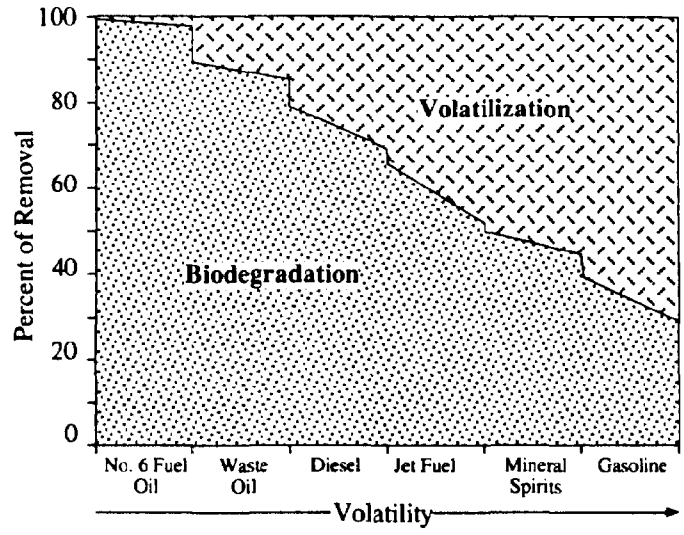

Figure 19. Removal mechanisms as a function of product volatility.

water from the sparge area. To prevent these undesired effects, sparge systems should be operated in conjunction with a vent system that effectively captures sparged gases, and at injection rates which cause minimal disturbance of hydraulic gradients in the injections area.

As with any technology, there are limitations to the utility and applicability of air sparging. For air sparging to effectively strip contaminants from ground water, the contaminant must be relatively volatile and relatively insoluble. If air sparging is used to supply oxygen for biodegradation, the contaminant should be soluble, relatively nonvolatile, and relatively biodegradable. The geological characteristics of the site, most importantly heterogeneity, also limit the applicability of air sparging. Changes in lithology can profoundly affect both the direction and velocity of air flow. If significant stratification is present, sparged air could be held below an impervious layer and spread laterally, thereby resulting in the spread of contamination (**Figure 20**).

As shown in **Figure 21**, highly permeable zones may lead to rapid and unpredicted movement of air containing volatilized contaminants. Another constraint of concern is depth related. There is both a minimum and maximum depth for a sparge system. A minimum depth of 4 feet, for example, may be required to confine the air and force it to "cone-out" from the injection point. A maximum depth of 30 feet might be required from the standpoint of control. Depths greater than 30 feet make it difficult to predict where the sparged air will travel.

4.3 Bioventing

Bioventing is the process of supplying air or oxygen to soil to stimulate the aerobic biodegradation of contaminants (Hinchee, in press). Bioventing is a modification of the technology variously referred to as soil vacuum extraction, vacuum extraction, soil gas extraction, or in-situ volatilization. Bioventing systems are generally operated at lower air flow rates than soil vacuum extraction systems to minimize extraction of volatile contaminants and maximize contact time between soil microbes and injected air. Soil bioventing is applicable to remediation of contaminants of low

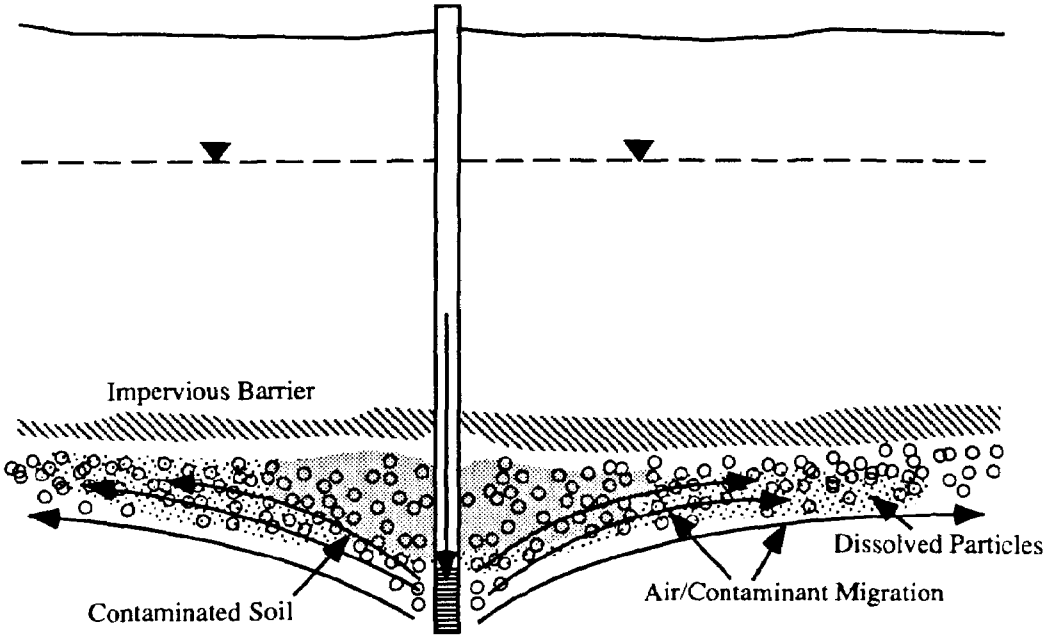

Figure 20. Inhibited vertical air flow due to impervious barrier.

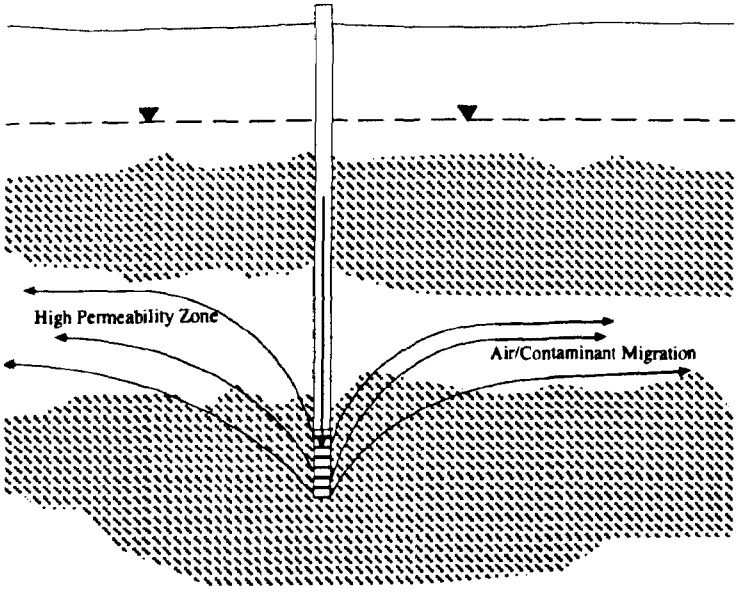

Figure 21. Preferential air flow through highly permeable zones (Brown, in press).

volatility and can also reduce concentrations of volatile contaminants in off-gases, thus reducing the amount of contaminants requiring off-gas treatment.

The primary advantage of using air instead of water to transport oxygen to the subsurface is that the oxygen content of air is 21%, or 21,000 parts per million. The amount of air required to satisfy high oxygen demands during biodegradation is, therefore, much lower than the amount of oxygen-saturated water. In addition, air is more easily moved through the subsurface, whereas hydraulic limitations when using water may impede delivering necessary amounts of oxygen. Clean air may be injected directly into the contaminated zone by injection wells or extracted with vacuum extraction wells. **Figure 22** shows two possible configurations of an in-situ bioventing system.

Bioventing is primarily applicable to contaminants in the vadose zone, but can also be applied to saturated zones which have been dewatered (**Figure 23, dark shaded region**).

Laboratory research and field demonstrations involving bioventing began in the early 1980s, with particular emphasis to the remediation of soil contaminated with hydrocarbons. A detailed history is given by Hinchee (in press). Early on, researchers concluded that venting would not only remove gasoline by physical means but would also enhance microbial activity and promote the biodegradation of gasoline (Texas Research Institute, 1980; 1984). The first actual field-scale bioventing experiments were conducted by van Eyk for Shell Oil (Hinchee, in press). A series of experiments were conducted by Delft Geotechnics to investigate the effectiveness of bioventing for treating hydrocarbon-contaminated soils, and reported in a series of papers (Anonymous, 1986; Staatsuitgeverij, 1986; van Eyk and Vreeken, 1988, 1989a, and 1989b).

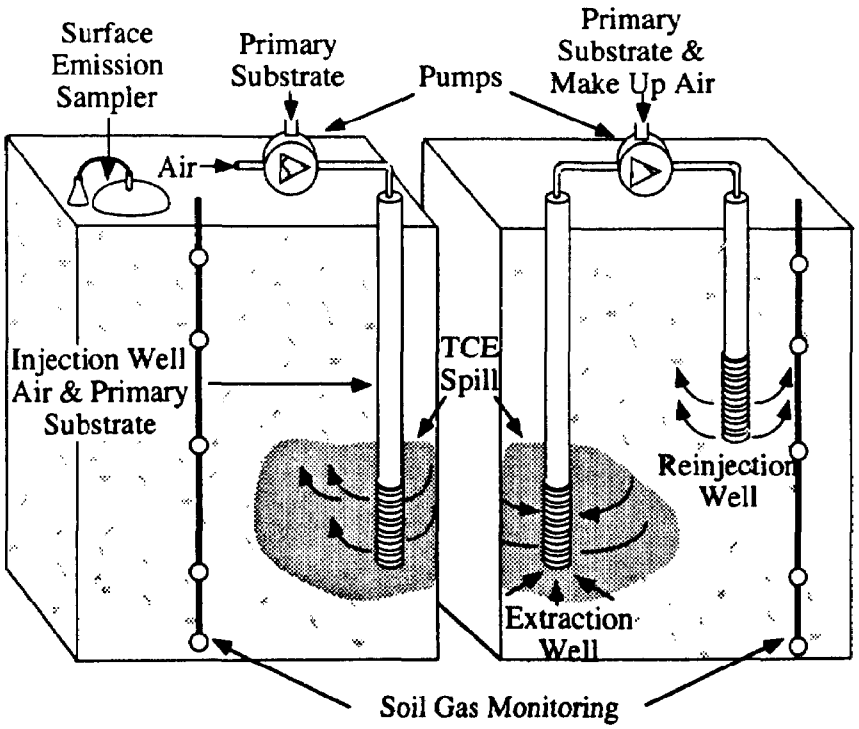

Figure 22. Two configurations of bioventing. Left - air injection. Right - vacuum extraction with air reinjection (Wilson and Kampbell, in press).

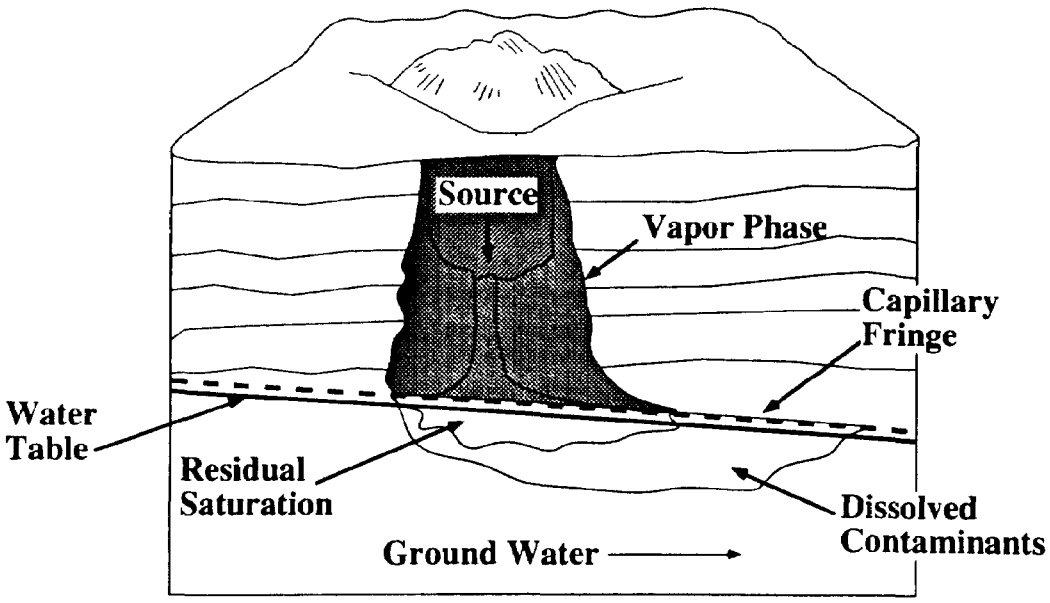

Figure 23. Contaminant locations treatable with bioventing.

Bioventing is potentially applicable to any contaminant that is more readily biodegradable aerobically than anaerobically. Although most applications have been to petroleum hydrocarbons, applications to PAH, acetone, toluene, and naphthalene mixtures have been reported. In most applications, the key is biodegradability versus volatility. If the rate of volatilization significantly exceeds the rate of biodegradation, removal essentially becomes a volatilization process. In general, low-vapor pressure compounds (less than 1 mm Hg) cannot be successfully removed by volatilization and can only be biodegraded in a bioventing application. Higher vapor pressure compounds (above 760 mm Hg) are gases at ambient temperatures and therefore volatilize too rapidly to be biodegraded in a bioventing system. Within this intermediate range (1-760 mm Hg) lie many of the petroleum hydrocarbon compounds of regulatory interest, such as benzene, toluene, and the xylenes, that can be treated by bioventing.

In addition to the normal site characterization required for the implementation of this or any other remediation technology, additional investigations are necessary. Soil gas surveys are required to determine the amount of contaminants, oxygen, and carbon dioxide in the vapor phase; the latter are needed to evaluate in-situ respiration under site conditions. An estimate of the soil gas permeability, along with the radius of influence of venting wells, is also necessary to design full-scale systems, including well spacing requirements, and to size blower equipment.

Although bioventing has been performed and monitored at several field sites, many of the effects of environmental variables on bioventing treatment rates are still not well understood (Hinchee, in press). In-situ respirometry at additional sites with drastically different geologic conditions has further defined environmental limitations and site-specific factors that are pertinent to successful bioventing. However, the relationship between respirometric data and actual bioventing treatment rates has not been clearly determined. Concomitant field respirometry and closely monitored field bioventing studies are needed to determine the type of contaminants that can successfully be treated by in-situ bioventing and to better define the environmental limitations to this technology.

SECTION 5
CONTAMINANT BIODEGRADABILITY

There is considerable information in the literature considering the biodegradability of organic contaminants. However, extrapolation of this information to site-specific, full-scale bioremediation systems is quite difficult. This requires that additional testing be done to establish that the contaminants of concern actually will biodegrade under prevailing site conditions. Determination of biodegradability has traditionally been done first at the laboratory scale, followed by bench- and field-scale studies.

5.1 Laboratory Testing

Laboratory tests can be used as screening tests to determine site feasibility, as treatability tests to determine the rate and extent of biodegradation that might be attained during remediation, and as engineering tests to provide design criteria (U.S. EPA, 1991b). Screening tests include pH and microbial plate counts to determine if existing conditions are favorable to microbial growth. Respirometer tests, which measure oxygen uptake but do not normally measure disappearance of the contaminant(s), provide confirmation that the microbial population is metabolically active. These tests can be run under a number of nutrient conditions to provide an indication of nutrient effects. Laboratory scale tests are often used as "proof-of-concept" screens, rather than to obtain information to be used in designing full-scale systems.

Laboratory treatability studies are generally conducted with soil/ground-water slurries (flask studies) or soils (pan studies). Several conditions are usually tested, including unmodified microcosms, nutrient amended microcosms, and biologically inhibited conditions. These tests can measure the rate of change of the constituents of concern as well as changes in pH and microbial populations. The tests provide data on the rate and extent of conversion of contaminants. During bioremediation of hydrocarbons in aquifers, the rate of degradation is usually controlled by the rate of supply of nutrient and oxygen. Under these conditions, laboratory rate data do not extrapolate directly to the field. However, laboratory data on the rate and extent of removals of hazardous constituents are important for the heavier hydrocarbons, such as heavy crude oil, bunker oil, or coal gas tars. Removal of compounds from these materials is often limited by the reaction kinetics of the microorganisms rather than the rate of supply of some essential nutrient. The extent of biodegradation of oily phase hydrocarbons to microbial biomass or metabolic end products is very site specific.

5.2 Field Testing: Pilot and Full Scale

Demonstration that a contaminant is biodegradable under laboratory conditions does not automatically mean that bioremediation will be successful under full-scale field conditions. Many variables such as site hydrology and geochemistry, contaminant nature and distribution, and predominant environmental conditions may limit the extent of biodegradation. Because of these factors, pilot testing of proposed bioremediation systems is almost always necessary.

5.3 Petroleum Hydrocarbons

As a class, petroleum hydrocarbons are biodegradable and can generally be mineralized, i.e., converted to carbon dioxide and water. The rate and extent of hydrocarbon biodegradation in the subsurface will depend on several factors, including (1) the quantity and quality of nutrients and electron

acceptors; (2) the type, number and metabolic capability of the microorganisms; and (3) the composition and amount of the hydrocarbons. While virtually all petroleum hydrocarbons are biodegradable, the rate and extent of biodegradation can be highly variable. Depending on environmental conditions, biodegradation may be very rapid or very slow. The ease of biodegradation depends somewhat on the type of hydrocarbon. As molecular weight increases, so does the resistance to biodegradation. The lighter, more soluble members are generally biodegraded more rapidly and to lower residual levels than are the heavier, less soluble members. Moderate to lower molecular weight hydrocarbons (C_{10} to C_{24} alkanes, single ring aromatics) appear to be the most easily degradable hydrocarbons (Atlas, 1988). Thus, monoaromatic compounds such as benzene, toluene, ethylbenzene, and the xylenes are more rapidly degraded than the two-ring compounds such as naphthalene, which are in turn more easily degraded than the three-, four-, and five-ring compounds. The same is true for aliphatic compounds where the smaller compounds are more readily degraded than the larger compounds. Branched hydrocarbons degrade more slowly than the corresponding straight chain hydrocarbons.

Because petroleum hydrocarbons are frequently found in the presence of other organic constituents, it is necessary to consider the degradability of other classes of compounds. Non-chlorinated solvents used in a variety of industries are generally biodegradable. For example, alcohols, ketones, esters, carboxylic acids and esters (particularly the lower molecular weight analogs) are readily biodegradable but may be toxic at high concentrations due to their high water solubilities. It is reasonable to expect that some aerobic biodegradation of chlorinated solvents will occur in the presence of petroleum hydrocarbon blends, particularly those containing appreciable amounts of toluene. This is, however, a very site-specific phenomenon and one for which there is not enough documentation to make reliable predictions. Further, many chlorinated solvents can inhibit biodegradation of petroleum hydrocarbons.

Site remediation is usually concerned with commercial blends of petroleum hydrocarbons, such as gasoline and other fuels. As for individual compounds, the lighter blends are more readily degraded than the heavier blends. The extent of conversion that is likely to occur is greatest for lower molecular weight constituents. Gasolines contain primarily low to moderate molecular weight compounds and can be biodegraded to low levels under many conditions. For gasoline, the extent of conversion is largely limited by the efficiency and completeness of the distribution of nutrients and an electron acceptor. Heavier products such as Number 6 fuel oil or coal tar, however, contain many higher molecular weight compounds such as five-ring polyaromatic compounds. These mixtures degrade much more slowly than gasoline and, as a result, significantly lower rates and extent of biodegradation should be anticipated.

5.3.1 Fuels

Gasoline is a complex mixture of hydrocarbons blended from various refinery products. The specific composition is variable and depends on source of petroleum, individual refinery process streams used for blending, and customer specifications. The primary components of gasolines are volatile hydrocarbons which boil at temperatures below 200° C (390° F). Hydrocarbons in this boiling range have 4-12 carbon atoms in their molecular structure. In refinery terms these compounds are derived from light ends, referring to both the relatively low boiling points and the low molecular weight which typify these compounds. The major components of blended gasolines are branched-chain paraffins, cycloparaffins, and aromatic compounds (Cline et al., 1991). Composition of a typical leaded and unleaded gasolines is shown in **Table 1.** Gasolines also may contain a number of additives such as dyes, antiknock agents, lead scavengers, anti-oxidants, metal deactivators, octane enhancers, corrosion inhibitors, and oxygenates. Specific composition of gasoline will vary both regionally and seasonally. Daily changes in refinery operation may cause variations in product composition.

TABLE 1. COMPOSITION OF GASOLINES (FROM CLINE ET AL., 1991)

Compound	volume % in leaded fuel	volume % in unleaded fuel
Normal/iso hydrocarbons	59	55
isopentane	9-11	9-11
n-butane	4-5	4-5
Aromatic hydrocarbons	26	34
xylenes	6-7	6-7
toluene	6-7	6-7
ethylbenzene	5	5
benzene	2-5	2-5
napthalene	0.2-0.5	0.2-0.5
Olefins	10	5
Cyclic hydrocarbons	5	5

The most common dissolved hydrocarbons (benzene, toluene, ethylbenzene and xylenes) released from gasoline spills are known to be readily biodegradable under aerobic conditions (Jamison et al., 1975; Gibson and Subramanian, 1984; Thomas et al., 1990; Alvarez and Vogel, 1991). In addition, aerobic hydrocarbon degrading microorganisms are very common in nature and have been recovered from virtually all petroleum contaminated sites that have been studied (Litchfield and Clark, 1973). For most petroleum sites, extensive studies to confirm the presence of BTEX degrading microorganisms are probably not necessary. Jamison et al. (1975) found that the vast majority of gasoline components were readily degraded by a mixed microbial population obtained from a gasoline contaminated aquifer. Although many of the individual gasoline components would not support microbial growth as a sole carbon source, they did disappear when gasoline dissolved in water was used as the substrate. This suggests that a mixed microbial population may be necessary for complete degradation. In a study of the catabolic activity of bacteria from an aquifer contaminated with unleaded gasoline, Ridgeway et al. (1990) found that most isolates were very specific in their ability to degrade hydrocarbons. Although all of the 15 hydrocarbons tested were degraded by at least one isolate, most organisms were able to degrade only one of several closely related compounds. Toluene, *p*-xylene, ethylbenzene, and 1,2,4-trimethylbenzene were most frequently utilized whereas cyclic and branched alkanes were least utilized.

In contrast to BTEX, there is much less information available on the biodegradability of many fuel additives such as methyl tertiary butyl ether (MTBE), 1,2-dibromoethane (EDB), or 1,2-dichloroethane (EDC). MTBE is of special concern because it is extremely water soluble, is used as an oxygenate in fuels at levels up to 15%, and is not known to biodegrade. If persistence of fuel additives is a concern, site specific studies may be needed to confirm the presence of microorganisms capable of degrading these compounds and to estimate biodegradation rates.

5.3.2 PAHS (Coal Tar, Creosote, Refinery Wastes)

Polyaromatic hydrocarbons (PAHs) are present in heavier petroleum hydrocarbon blends and particularly in coal tars, wood treating chemicals, and refinery wastes. PAHs have only limited solubility in water, adsorb strongly to subsurface materials, and degrade at rates much slower than monoaromatic hydrocarbons or most aliphatic and alicyclic compounds found in refined petroleum hydrocarbon products. As a result, they often persist for long time periods even under ideal conditions (Lee, 1986; Borden et al., 1989). Because of their low solubility and strong adsorption to solids, their availability for degradation is often the limiting factor in treatment (Brubaker, 1991). For the heavier petroleum hydrocarbons, especially PAHs, the limiting factors may be rate of solubilization, release from interstitial pore spaces, or rate of degradation of these higher molecular weight constituents. They are more likely to be biodegraded in mixtures with more soluble and thus more readily degradable hydrocarbons because the more readily degradable species will support a larger microbial population (McKenna and Heath, 1976). The higher molecular weight PAHs with high numbers of aromatic rings (e.g., benzo (a) pyrene) have the slowest rates of biodegradation. This is due in part to very low solubility and high sorption tendency. In addition, the complex molecular structure of these compounds makes them resistant to attack by a single organism, and many of the 5- and 6-ring compounds are only biodegradable through cometabolic processes. Unfortunately, many of these compounds are of regulatory concern because of cancer-causing tendencies. Thus, the least biodegradable PAHs are often regulated to very low treatment goals.

5.4 Chlorinated Solvents

Chlorinated solvents and their natural transformation products represent the most prevalent organic ground-water contaminants in the country. These solvents, consisting primarily of chlorinated aliphatic hydrocarbons (CAHs), have been widely used for degreasing aircraft engines, automotive parts, electronic components, and clothing. Because of their relative solubility in water and their somewhat poor sorption to soils, they tend to migrate downward through soils, contaminating water with which they come into contact. Being denser than water, their downward movement is not impeded when they reach the water table, and so they can penetrate deeply beneath the ground-water table. CAHs have water solubilities in the range of 1 g/l, or several orders of magnitude higher than the drinking water standards for those that are regulated.

The major chlorinated solvents used in the past are carbon tetrachloride (CT), tetrachloroethene (PCE), trichloroethene (TCE), and 1,1,1-trichloroethane (TCA). These compounds can be transformed by chemical and biological processes in soils to form a variety of other CAHs, including chloroform (CF), methylene chloride (MC), *cis*- and *trans*-1,2-dichloroethene (*cis*-DCE, *trans*-DCE), 1,1-dichloroethene (1,1-DCE), vinyl chloride (VC), 1,1-dichloroethane (DCA), and chloroethane (CA). These chemicals, their solubilities in water, and drinking water maximum contaminant limits, if applicable, are listed in **Table 2**. This is the group of chemicals generally to be addressed as a result of chlorinated solvent contamination of ground water.

Fifteen years ago, many of these highly chlorinated organic compounds were considered recalcitrant to biological degradation in the environment. Transformation products of the chlorinated solvents then started to be found in ground waters, and this led to expanded efforts to determine the chemical and biological processes responsible. It was found that most of the CAHs can in fact be transformed by biological processes; but generally, the microorganisms responsible cannot obtain energy for growth from the transformations. The transformations are most often brought about by cometabolism,

TABLE 2. COMMON CHLORINATED ALIPHATIC HYDROCARBON (CAH) CONTAMINANTS IN GROUND WATER (MCCARTY AND SEMPRINI, IN PRESS)

Compound	Formula	Acronym	Density	Water Solubility (mg/l)	U.S. Drinking Water MCL (µg/l)
Carbon tetrachloride	CCL_4	CT	1.595	800	5
Chloroform	$CHCL_3$	CF	1.485	8,200	100
Methylene chloride	CH_2CL_2	MC	1.325	13,000	-
1,1,1-trichloroethane	CH_3CCL_3	TCA	1.325	950	200
1,1-dichloroethane	CH3CHCL2	1,1-DCA	1.175	5,500	-
1,2-dichloroethane	CH_2CLCH_2CL	1,2-DCA	1.253	8,700	5
chloroethane	CH_3CH_2CL	CA	-	-	-
Tetrachloroethene	CCL_2=CCL_2	PCE	1.625	150	5
Trichloroethene	CHCL=CCL_2	TCE	1.462	1,000	5
cis-1,2-dichloroethene	CHCL=CHCL	*cis*-DCE	1.214	400	70
trans-1,2-dichloroethene	CHCL=CHCL	*trans*-DCE	1.214	400	100
1,1-dichloroethene	CH_2=CCL_2	1,1-DCE	-	-	7
Vinyl chloride	CH=CHCL	VC	-	-	2

through interactions of the CAHs with enzymes or cofactors produced by the microorganisms for other purposes.

Microbially-mediated reactions of chlorinated solvents usually involve oxidation or reduction reactions. Oxidation reactions are generally slower with highly halogenated compounds than with compounds containing fewer halogen substituents, while the opposite is true for reduction reactions. Oxidation reactions do not dehalogenate in the first, rate-limiting step, but in subsequent steps. Reduction reactions normally include the dehalogenation of these solvents, producing less halogenated homologues. The dechlorination occurs under anaerobic conditions and results in less chlorinated, and often aerobically degradable, products. Engineered systems, or in-situ bioremediation, can effectively employ either aerobic alone or sequential anaerobic/aerobic microbial processes to biodegrade chlorinated solvents.

There are now widespread efforts to take advantage of cometabolism for the transformation of CAHs in ground water; but this is a much more complicated process than the usual biological treatment processes that have been used for years, in which organic compound destruction is accomplished by organisms that use the compounds as primary substrates for energy and growth. In cometabolism, other chemicals must be present to serve as primary substrates to satisfy the energy needs of the microorganisms. These substrates must be carefully selected to stimulate the production of the enzymes that affect cometabolism of the CAHs.

Much has already been learned about cometabolism of CAHs. However, full-scale field applications of this process are greatly limited; and there are virtually no sufficiently well-documented full-scale applications at present that can be used to guide design and application or that can be used to evaluate costs. Thus, any application of bioremediation for chlorinated solvent destruction in the field must be considered as a research activity and should be evaluated as such. As with any new and untested

process, failure to reach desired goals should be anticipated; and surprises can be expected. Nevertheless, the understanding of the process is now at a stage where full-scale experimentation is desirable and indeed is a necessity if biodegradation of chlorinated solvents is to become a reality rather than just a laboratory curiosity.

5.4.1 Aerobic Biodegradation of CAHs

Several of the common chlorinated solvents (chlorinated ethanes and ethenes) can be degraded under aerobic conditions (Norris, in press). Lightly chlorinated compounds such as chlorobenzene (U.S. EPA, 1986), dichlorobenzene, chlorinated phenols and the lightly chlorinated PCBs are also typically biodegradable under aerobic conditions. Highly chlorinated organic compounds are much more oxidized than many natural organics. As such, these compounds do not provide much energy upon further oxidation in aerobic environments. Most aerobic biodegradation processes start with a step that involves the insertion of oxygen into a bond on the molecule. Due to the electrophilic nature of that oxygen insertion, other electrophilic substituents (e.g., chlorine) hinder the reaction. Hence, the observation that increasing chlorination within a homologous series often leads to a decrease in aerobic (oxidative) biodegradation (Vogel et al., 1987). These more highly chlorinated analogs are more recalcitrant to aerobic degradation but are more susceptible to degradation under anaerobic conditions.

Most of the research to date has described the microbial oxidations of mono- or dihalogenated aliphatic compounds. The major exception to this is the work done on the oxidation of trichloroethylene (TCE). Several different microbes or microbial enrichments have been shown to be capable of TCE oxidation (Fogel et al., 1986; Nelson et al., 1986; Little et al., 1988) and chloroform oxidation (Strand and Shippert, 1986). Apparently, the ease of oxidation increases as the number of halogens decreases. Hence, dichloroethylene would be oxidized faster than TCE. Unfortunately, due to the nature of contaminant release in the environment, mass balances are difficult to achieve; and no strong evidence for the oxidation of halogenated solvents has been derived from actual hazardous waste sites.

Studies of the aerobic biodegradation of chlorinated compounds have illustrated several major pathways of oxidation. These pathways resemble those for the nonchlorinated homologs. For example, the oxidation of chlorinated ethylenes involves the formation of a chlorinated epoxide which degrades rapidly in water. This is similar to the epoxide formed from ethylene. In both of these cases, the microbe that degrades these compounds might require the addition of a natural nonchlorinated compound, or **cometabolite** for growth and energy. The enzymes produced for degradation of that "normal" substrate are also capable of degrading the pollutant (cometabolism). Certain contaminants such as toluene or phenol, if present with the chlorinated species, can act as cometabolites. Although early work indicated that some CAHs, particularly those with few chlorines on the molecule, were biodegradable by microorganisms, knowledge that a broader range of CAHs can be oxidized aerobically through cometabolism is rather recent. Wilson and Wilson (1985) showed for the first time that TCE may be susceptible to aerobic degradation through use of soil microbial communities fed natural gas. These **methanotrophs** use an oxygenase (methane monooxygenase or MMO) to catalyze the oxidation of methane to methanol. MMO also oxidizes TCE fortuitously to form TCE epoxide (Little et al., 1988; Fox et al., 1990), an unstable compound that undergoes abiotic chemical decomposition to yield a variety of products, including carbon monoxide, formic acid, glyoxylic acid, and a range of chlorinated acids (Miller and Guengerich, 1982). In mixed cultures as in nature, cooperation between the TCE oxidizers and other bacteria occurs; and TCE is further mineralized to carbon dioxide, water, and chloride (Fogel et al., 1986; Henson et al., 1989; Roberts et al., 1989; Henry and Grbić-Galić, 1991a).

Since the report of Wilson and Wilson (1985) on TCE cometabolism, much scientific research addressing this phenomenon has been performed. The groups of aerobic bacteria currently recognized as being capable of transforming TCE and other CAHs through cometabolism comprise not only the methane oxidizers (Fogel et al., 1986; Little et al., 1988; Mayer et al., 1988; Oldenhuis et al., 1989; Tsien et al., 1989; Henry and Grbić-Galić, 1990; Alvarez-Cohen and McCarty, 1991a,b; Henry and Grbić-Galić, 1991a,b; Lanzarone and McCarty, 1991; Oldenhuis et al., 1991), but also propane oxidizers (Wackett et al., 1989), ethylene oxidizers (Henry, 1991), toluene, phenol, or cresol oxidizers (Nelson et al., 1986, 1987, 1988; Wackett and Gibson, 1988; Folsom et al., 1990; Harker and Kim, 1990), ammonia oxidizers (Arciero et al., 1989; Vannelli et al., 1990), isoprene oxidizers (Ewers et al., 1991), and vinyl chloride oxidizers (Hartmans and de Bont, 1992). These microorganisms all have catabolic oxygenases that catalyze the initial step in oxidation of their respective primary or growth substrates and have potential for initiating the oxidation of CAHs.

There is currently insufficient information on the relative advantages and disadvantages of the different oxygenase systems to recommend definitively one over the other, but each may have its place. Most research to date has been conducted with the methane oxidizers and the group of bacteria containing toluene oxygenase, which can be induced with primary substrates such as toluene, phenol, and cresol. The oxygenases for the above organisms are often nonspecific and fortuitously initiate oxidation of a variety of compounds including most of the CAHs. The exceptions are highly chlorinated CAHs such as CT and PCE. In general, oxygenases act on unsaturated CAHs such as TCE by adding oxygen across the double bond to form an epoxide. With saturated CAHs, such as CF or TCA, a hydroxyl group is generally substituted for one of the hydrogen atoms in the CAH molecule. Frequently, the resulting products from CAH oxidation are chemically unstable and decompose as described above for TCE, yielding products that are further metabolized by other microorganisms present in nature.

In other aerobic degradation pathways of lightly chlorinated compounds, microbes have been shown to grow on the pollutant when it exists in sufficiently high concentration. Most of these compounds are mono- or dichlorinated organics. A common reaction is the microbially mediated substitution reaction where a hydroxyl group replaces a chlorine (Brunner et al., 1980). After this, the compound is further oxidized; and the metabolites enter the anabolic and catabolic pathways of the microbe. The possibility that these microbes would adapt to the use of chlorinated compounds as sources of energy and carbon exists; but this might have limited engineering applications, as will be discussed later. Selective pressure in natural environments will not be great if pollutant concentrations are relatively low from a microbial adaptation point of view, even if these concentrations are high from a regulatory point of view.

5.4.2 Anaerobic Biodegradation of CAHs

As noted above, many highly chlorinated compounds are resistant to aerobic degradation due to their highly oxidized state. Examples of chlorinated organics resistant to aerobic degradation include (1) tetrachloroethylene, which has not been observed to undergo epoxidation and (2) hexachlorobenzene, which has all carbons occupied with chlorine substituents, allowing no site for hydroxylation. These highly chlorinated organic compounds are not, however, resistant to anaerobic biodegradation (Vogel and McCarty, 1985; Gibson and Suflita, 1986; Tiedje et al., 1987; Vogel and McCarty, 1987a; Vogel, 1988; Freedman and Gossett, 1989; Bagley and Gossett, 1990; Nies and Vogel, 1990; Bhatnagar and Fathepure, 1991). Several studies provide evidence for anaerobic transformation of chlorinated solvents by pure cultures of bacteria. The bacteria involved ranged from strict anaerobic microorganisms, such as methanogens, sulfate-reducers, and clostridia to facultative anaerobes such as *Escherichia coli* or *Pseudomonas putida*. Reductive dechlorination was the predominant reaction pathway. Consequently, the

chlorinated solvent biotransformation studies with environmental samples (mixed microbial cultures) and pure bacterial cultures indicate that a broad variety of bacteria possess the enzymatic capability to reductively dechlorinate the compounds. An electron donor, such as low molecular weight organic compounds (lactate, acetate, methanol, glucose, etc.) or H_2, must be available to provide reducing equivalents for reductive dechlorination. Toluene was recently found to be a suitable electron donor for the reductive dechlorination of PCE to DCE in anaerobic aquifer microcosms (Sewell and Gibson, 1991).

Anaerobic biotransformation of chlorinated solvents has been observed in field studies (Roberts et al., 1982), in continuous-flow fixed-film reactors (Bouwer and McCarty, 1983b; Vogel and McCarty, 1985, 1987; Bouwer and Wright, 1988), and in soil (Kloepfer et al., 1985), sediment (Barrio-Lage et al., 1986), and aquifer microcosms (Wilson, B. et al., 1986) under conditions of denitrification, sulfate reduction, or methanogenesis. Table 3 illustrates commonly observed anaerobic biotransformations. The initial step in the anaerobic biotransformation was generally reductive dechlorination. For example, CF was produced from CT, and 1,1-dichloroethane (1,1-DCA) was produced from 1,1,1-TCA.

The transformations of PCE and TCE have been studied most intensely. General agreement exists that transformation of these two compounds under anaerobic conditions proceeds by sequential reductive dechlorination to dichloroethene (DCE) and vinyl chloride (VC), and in some instances, there is total dechlorination to ethene or ethane. Of the three possible DCE isomers, 1,1-DCE is the least significant intermediate. Several studies have reported that *cis*-1,2-DCE predominates over *trans*-1,2-DCE (Barrio-Lage et al., 1986; Parsons et al., 1984; Parsons and Lage, 1985). CT, CF, 1,2-DCA, 1,1,1-TCA, and PCE were partially converted to carbon dioxide during anaerobic biotransformations. Reductive dechlorination of 1,1,1-TCA and PCE occurred first, prior to mineralization to carbon dioxide. Most of the experiments were conducted under methanogenic conditions. Several of the chlorinated compounds were also transformed by similar pathways under conditions of denitrification and sulfate reduction (**Table 3**).

TABLE 3. ANAEROBIC TRANSFORMATION OF SELECTED CHLORINATED SOLVENTS IN MICROCOSMS AND ENRICHMENT CULTURES UNDER DIFFERENT REDOX CONDITIONS (BOUWER, IN PRESS)

Chlorinated solvent[a]	Redox condition[b]	Transformation[c]	Intermediate[d]	End product	System	Refs[e]
CT	dn	+	CF	n.d.	biofilm reactor	d,e
	sr	+	CF	n.d	biofilm reactor	e
	me	+	CF	CO_2	biofilm reactor/ aquifer material	c,e,n
CF	dn	--	--	--	biofilm reactor	d,e
	sr	--	--	--	biofilm reactor	e
	me	+	n.d.	CO_2	biofilm reactor	c,e
1,2-DCA	me	+	n.d.	CO_2	biofilm reactor	c
1,1,1-TCA	dn	--	--	--	biofilm reactor	d,e,j
	sr	+	1,1-DCA	CA	biofilm reactor/ aquifer material	e,j
	me	+	1,1-DCA	CO_2	biofilm reactor/ aquifer material	c,e,j,r
1,1,2,2-TeCA	me	+	--	1,1,2-TCA	biofilm reactor	c
HCA	ae	+	--	PCE	aquifer material	f
	dn	+	n.d.	n.d.	biofilm reactor	e
	sr	+	n.d.	n.d.	biofilm reactor	e
	me	+	n.d.	n.d.	biofilm reactor	e
1,1-DCE	me	+	VC	n.d.	sediment/aquifer material	b,s
cis-1,2-DCE	me	+	VC	n.d.	sediment/aquifer material	b,s
trans-1,2-DCE	me	+	CA + VC	n.d.	sediment/aquifer material	b,s
TCE	me	+	*cis* -1,2-DCE/	n.d.	aquifer material	m,n
			trans-1,2-DCE1,2-DCE	n.d.	aquifer material	k,s
PCE	sr	+	TCE	*cis*-1,2-DCE	sewage sludge	a
	me	+	TCE	CO_2	biofilm reactor	q
				ethene	sewage sludge	g,h
				cis + *trans*-	aquifer material	p
				1,2-DCE	aquifer material/	i,o
				cis-1,2-DCE	sewage sludge	c,m,n,l
				n.d.	aquifer material	

[a] Abbreviations stand for: CT = carbon tetrachloride; CF = chloroform; DCA = dichloroethane; TCA = trichloroethane; CA = chloroethane; TeCA tetrachloroethane; HCA = hexachloroethane; DCE = dichloroethene; TCE = trichloroethene; PCE = tetrachloroethene.

[b] ae = aerobic; dn = denitrification; sr = sulfate reduction; me = methanogenesis.

[c] + = transformation observed; -- = no transformation

[d] n.d. = not determined

[e] a = Bagley and Gossett, 1990; b = Barrio-Lage et al., 1986; c = Bouwer and McCarty, 1983a;d = Bouwer and McCarty, 1983b; e = Bouwer and Wright, 1988; f = Criddle et al., 1986; g = DiStefano et al., 1991; h = Freedman and Gossett, 1989; i = Käsmer, 1991; j = Klecka et al., 1990; k = Kloepfer et al., 1985; l = Parsons and Lage, 1985; m = Parsons et al., 1984; n = Parsons et al., 1985; o = Scholz-Muramatsu et al., 1990; p = Sewell and Gibson, 1991; q = Vogel and McCarty, 1985; r = Vogel and McCarty, 1987; s = Wilson et al., 1986a.

SECTION 6
MONITORING AND PERFORMANCE EVALUATION FOR BIOREMEDIATION SYSTEMS

One of the key elements in applying in-situ bioremediation systems is evaluation of performance. Several approaches have been used: (1) monitoring contaminant concentration in ground water (dissolved phase), (2) monitoring contaminant concentration in soils or aquifer material (residual saturation), and (3) monitoring changes in levels of electron acceptors and nutrients within and around the contaminated zone.

Demonstrating decreases in ground-water concentrations of contaminants of concern has been the most common approach. Unfortunately, the solubility of petroleum hydrocarbons is low; and most of the hydrocarbon mass is associated with the solids, not the dissolved phase. Since the mass of contaminant trapped at residual saturation within the soil or aquifer is the source of any hydrocarbons which are dissolved in ground water, quantities associated with aquifer solids are far more important than ground-water concentrations. Therefore, site characterization should include delineation of the extent of contaminant mass acting as a source. Performance can then be evaluated based on decreases in the total amount of contaminant, rather than the fraction which has dissolved in ground water. This, however, is difficult to do, and is often considered to be outside of the scope of many investigations.

Demonstrating decreases in the mass or concentration of specific contaminants of concern in soils or aquifers is a primary requirement of performance evaluation. As noted earlier, measuring ground water concentrations of contaminants may not accurately indicate the extent of biodegradation of the source. Obtaining core samples from the contaminated subsurface before, during, and after bioremediation efforts is often the only means of doing this. Methods selected for analysis of samples should look for specific compounds rather than groups of compounds. Nonspecific parameters such as Total Petroleum Hydrocarbon (TPH) can measure components that are not of interest (e.g., asphalt particles), do not measure the most volatile compounds, and can yield highly variable results as shown in studies where split samples have been sent to different laboratories (Anonymous, 1992).

6.1 Sampling Programs

Comprehensive site characterization is critical to proper evaluation of performance of bioremediation systems. The primary criteria for evaluating treatment is a decrease in the concentration of a contaminant of concern to below a defined treatment goal, usually regulatory in nature. Due to site heterogeneity and costs associated with sampling and analysis, uncertainties about total mass of contaminants and variability in contaminant concentrations often exist. The range of initial contaminant concentration is often not known. If the variability in contaminant concentration at the outset of the treatment is not known, it will be impossible to determine if contaminant concentrations at the end of treatment represent statistically significant decreases, or simply variation in the distribution of contaminants. Site characterization information is often not this detailed.

Sampling programs to monitor performance are also often limited and not designed to obtain statistically representative data. Although obtaining representative data is sometimes difficult, particularly for sites with heterogeneous conditions and/or multiple sources, it is critical to design sampling programs which collect adequate information to demonstrate biodegradation. Although more extensive sampling

programs may increase the cost of bioremediation, it is essential to note that, without adequate data, claims of success are often not defensible.

6.2 Indicators

One useful method for assessing biodegradation activity is to monitor changes in the concentration of inorganic compounds within the aquifer (Borden, in press). Biodegradation of contaminants will result in the removal of electron acceptors (oxygen, nitrate, and sometimes sulfate) and release of waste products (carbon dioxide, reduced iron and methane) in areas where microorganisms are most active. If field monitoring indicates depletion of electron acceptors (oxygen, nitrate and/or sulfate) or production of waste products (carbon dioxide, soluble iron, or methane) within a plume or contaminated interval, this is a good indication that one or more of the contaminants are being biodegraded. The major limitation of this approach is that it is not possible to determine which specific compounds are being degraded or to what extent.

Oxygen, carbon dioxide, and methane are easily measured in the field, especially in soil gas surveys. Depletion of oxygen in contaminated zones is caused primarily by bacterial respiration. Accumulation of carbon dioxide within and adjoining the contaminated areas is also indicative of bacterial respiration. However, direct interpretation of carbon dioxide concentrations is sometimes difficult because carbon dioxide can be released during dissolution of certain minerals (e.g., limestone). Thus carbon dioxide can come from sources other than bacterial degradation. Methane is produced only under anaerobic conditions and can be used to monitor extent of anaerobic degradation. However, methane can exist as the result of biodegradation of naturally occurring organic materials, and care should be taken to verify that methane detected is actually generated within the contaminated area.

Other indicators of biological activity include changes in redox potential and pH. Measurement of redox potential is relatively simple and can provide a good qualitative indicator of the overall oxidation-reduction status of the aquifer. Redox potential can be measured using a platinum electrode and a standard pH meter. In locations where the redox potential is negative, the ground water is strongly reduced, indicating significant bacterial decomposition. In areas where the redox potential is positive, the ground water is oxidizing, indicating that the contaminant plume has not reached this point or that bacterial degradation has not occurred. In most cases, redox potentials should not be used for precise calculations but as a qualitative indicator of environmental conditions within and outside the contaminant plume (Barcelona et al., 1989). The pH of the system can be monitored to evaluate the extent of bacterial respiration. Many by-products of bacterial degradation of contaminants are organic acids and tend to decrease the pH of the system. However, aeration and addition of nutrients, as well as certain types of bacterial metabolism (i.e., denitrification), can also increase the pH and interpretation of pH data should take these factors into account.

6.3 Meeting Treatment Goals

The ability of in situ bioremediation to meet relevant regulatory goals depends both on the specified cleanup levels and the limits of the technology (Norris, in press). Endpoints can be mandated by state or federal numeric criteria such as National Safe Drinking Water Act MCLs, or they can be based on site specific risk assessments. Cleanup standards and acceptable timeframes can vary significantly according to state , and the specific levels and schedules set for a given site often determine which specific technology is employed. Regulations that set levels at or below analytical detection limits or site background concentrations may preclude the use of bioremediation.

Under ideal conditions, in-situ bioremediation can reduce petroleum hydrocarbon levels to nondetectable levels (10 mg/kg) (Norris, in press). This is more easily obtained with the lighter blends in permeable and homogeneous formations where placement of injection and recovery wells (galleries, etc.) is unencumbered. Generally, for lighter petroleum blends, the hardest regulatory endpoint to meet is the benzene limit (Norris, in press). Although benzene is highly biodegradable, MCLs for benzene (5 ppb) are at least an order of magnitude lower than for other specific light hydrocarbon constituents. As a result, if the benzene endpoint can be reached, the level for the other components will most probably be met as well.

For heavier petroleum hydrocarbons, BTEX compounds may not be present in significant concentrations to be of concern (Norris, in press). Typically, TPH will be the target analysis to be met. Because it is a nonspecific analysis, using background TPH levels as the remediation goal can create difficulties in interpreting data and lead to misleading conclusions regarding the performance of the system. The heavier the petroleum mixture, the more probable it is that there will be residuals of very slowly degraded components. These components tend to have low water solubilities, which can limit their rate of degradation. If TPH is the only criterion, the measurements will not determine which petroleum hydrocarbons components have gone untreated. Compounds that are not of environmental concern may contribute to reported TPH values and thus complicate interpretation.

Polyaromatic hydrocarbons can be difficult to treat to the regulated levels. The MCLs for many of these compounds are low because they are suspected carcinogens. The rate of release of PAHs from subsurface solids may be too slow to support an active microbial population, and degradation rates may be impractically slow. Fortunately, the degradability of these compounds is better in mixtures containing lower molecular weight compounds found in many commercial petroleum products. Available data on the limits of PAH degradation under in-situ bioremediation conditions are limited and contradictory, and thus predictions of treatment limits are likely to be unreliable.

6.4 Bioremediation Limits

6.4.1 Concentration

The range of contaminant concentrations that are amenable to bioremediation depends on a number of factors. These are illustrated in **Figure 24.**

High concentrations of contaminants can cause toxicity to microbes essential for biodegradation. Toxicity can occur with petroleum hydrocarbons, some chlorinated solvents and with certain very soluble compounds such as alcohols. The concentration at which a compound is toxic will be to some extent site and contaminant specific, as microbial communities usually have substantial capacity to adapt. In addition, mixtures of contaminants may exert toxicity where single contaminants would not. For example, individual components of creosote (such as naphthalene) exert less toxicity by themselves than the mixture. Some data on approximate concentrations at which specific compounds are toxic, biodegradability, and other properties of environmental interest are available from several handbooks (Montgomery, 1991; Montgomery and Wilkom, 1990; Howard, 1989 and 1990; and Verschueren, 1983). However, it should be remembered that this information has been generated under a variety of conditions. Therefore, determining the extent of contaminant toxicity generally requires site-specific measurements.

Very high concentrations of contaminants also may create very high oxygen and/or nutrient demands. Meeting these demands might require excessively longer times and higher costs than other technologies (Piontek and Simpkin, 1992), especially in aquifers with low hydraulic conductivity.

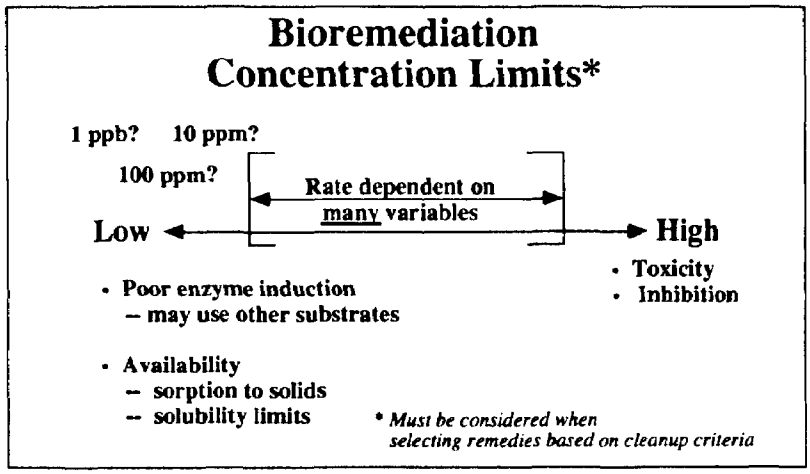

Figure 24. General concentration limits for bioremediation.

Although the contaminant may be amenable to in-situ bioremediation, in these situations it may be more practical to combine in-situ bioremediation with other technologies such as free-phase recovery, ground-water sparging, and in-situ vapor stripping.

Low contaminant concentrations also present limitations to bioremediation. First, certain concentrations of contaminant are required to induce biodegradation pathways in microbial populations. Below these concentration thresholds contaminants are often not degraded. Second, at low concentrations many compounds may not be available for microbial degradation. For example, high molecular weight PAHs such as benzo (a) pyrene have low water solubility and high sorption tendencies. At lower concentrations most of these types of contaminants will be bound to surfaces, and unavailable to microbial populations. Therefore, at concentrations considerably higher than some cleanup goals, biodegradation may either not occur at all or may stop before the target concentration is reached. Last, but not least, contaminant concentrations have to be high enough to support growth of the degrader populations. Contaminants concentrations which are above regulatory action levels may not be sufficient to support such growth.

6.4.2 Metals

Bioremediation is not generally applicable to metals-contaminated sites, but may mobilize or immobilize various metals. Generally, the presence of metals has little direct effect on the bioremediation process. While some metals such as zinc or mercury can be toxic to bacteria, the microbial population frequently adapts to the concentrations present. The effect of metals on the microbial population of a specific site must usually be tested through specific treatability studies. Bioremediation technologies for removal of metals from contaminated ground water are being developed but are still considered as emerging.

6.4.3 Mass Transfer

When large quantities of oily phase or viscous materials such as the heavier fuel oil blends are present, the flow of water through the contaminated zone may be impeded, preventing the delivery of nutrients and/or electron acceptors. In these situations, in-situ bioremediation is generally not feasible.

The concentration at which this occurs will vary with soil type, but generally will be above 20,000 mg/kg. In aquifers where NAPLs occur, both free-phase product and residual saturation may not be available for microbes to biodegrade. Research is in progress investigating the use of microbes which produce biosurfactants to overcome some of these barriers, but application of these in bioremediation systems is still considered to be in the developmental stages.

SECTION 7
STATE OF BIOREMEDIATION TECHNOLOGY

7.1 Natural Attenuation

At present, there are no well-documented full-scale demonstrations of natural bioremediation, although there has been some limited research into the processes that control the natural biodegradation of dissolved hydrocarbon plumes (Borden et al., 1986; Barker et al., 1987; Franks, 1987; Hult, 1987a; Chaing et al., 1989; Wilson et al, 1992). In addition, there is almost no operating history to judge the effectiveness of natural bioremediation. Early attempts at aquifer remediation focused on using conventional remediation techniques to remove or permanently immobilize contaminants at the highest priority sites. At many low priority sites, regulators have assumed that natural bioremediation would be adequate to control migration of dissolved contaminants. However, these sites have typically not been monitored sufficiently to determine if this approach is actually effective or to identify those factors that influence the efficiency of natural bioremediation. The primary repositories of expertise on natural bioremediation are in universities, in industry, the U.S. Environmental Protection Agency (U.S. EPA) and the U.S. Geological Survey (U.S.G.S.).

7.2 In-situ Bioremediation

In-situ bioremediation technologies are in varying stages of development. Although there has been considerable experience with laboratory and field-scale systems, information on full-scale application of bioremediation is still relatively limited. The technology, in general, is applicable and practicable. In-situ bioremediation of petroleum hydrocarbons is fairly well established. However, considerable expertise is required along with detailed knowledge of site hydrogeology, contaminant nature and distribution, and desired microbial metabolism. In addition, difficulties in evaluating performance hamper efforts to establish the success of various applications.

Information on commercial application of in situ bioremediation has recently been compiled by the USEPA Office of Research and Development (USEPA, 1992b). Detailed information concerning these case studies is maintained on the ATTIC (Alternative Treatment Technology Information Center) electronic bulletin board database. Vendors supplied information on 132 case studies. Full-scale systems were reported for 86 of these studies. Approximately 85% of the systems reported were for petroleum related, wood-preserving wastes, or solvents.

7.2.1 Anaerobic Bioremediation—Alternative Electron Acceptors

The demonstration of nitrate-based bioremediation in the field is limited; therefore, the use of this alternate electron acceptor for bioremediation must be viewed as a developing treatment technology. **Table 4** summarizes results of selected field studies where denitrification was tested as a means to remove aromatic hydrocarbon contamination. The Traverse City and the Rhine River Valley studies involved actual contamination sites. The Borden experiment involved injection of a mixture containing benzene, toluene, and xylene isomers (BTX) in one experiment and gasoline in another. In general, BTX compounds were found to disappear within the nitrate amended zone. Interpretation of these data, however, was complicated by a number of factors, especially the co-occurrence of nitrate and O_2, and the lack of complete characterization of the organic substrates. Nitrate removal exceeded the expected

TABLE 4. FIELD STUDIES WHERE DENITRIFICATION HAS BEEN EVALUATED (REINHARD, IN PRESS)

Study Site and Authors	Contamination and Conditions	Major Implication for In-Situ Bioremediation
Traverse City, MI, Hutchins and Wilson, 1991	JP-4 fuel, $NaNO_3$: 62 mg/l, O_2: 0.5 to 1 mg/l	(1) Removal of benzene, toluene, *m,p*-xylene; Recalcitrance of *o*-xylene. (2) Nitrate removed exceeded stoichiometric amount of BTEX removal. (3) Partitioning of compounds into the water phase appears to be a major factor determining compound removal.
Borden, Ontario, Berry-Spark et al., 1988	Gasoline and BTX; Oxygen and nitrate	(1) BTX transform more slowly when gasoline is present than in systems where BTX are the only substrate. (2) In systems containing both O_2 and nitrate, aerobic and (facultative) denitrifying organisms appear to cooperate.
Seal Beach, Reinhard et al. 1991	Gasoline contaminated ground water feed, NO_3- (6 mg/l)	90% nitrate removal in mixed nitrate/sulfate system, aromatics removal toluene> *p*-xylene>*o*-xylene>benzene.
Rhine Valley, FRG, Werner, 1985	Fuel oil (?) Aerated water (O_2), NO_3^- (>300 mg/l), PO_4 (>0.3 mg/l), NH_4^+ (>1.0 mg/l)	(1) Removal fastest for benzene, slower for toluene, slowest for *p*-xylene. (2) Oxygen suspected to be electron acceptor initiating the transformation.

amount based on the substrates analyzed, and this was attributed to the dissolved organic carbon in ground water (Berry-Spark et al., 1988).

Bioremediation of chlorinated solvents using alternate electron acceptors is a developing treatment technology that is mostly being investigated at the laboratory scale. Limited field experience exists on stimulation of anaerobic biotransformation for control of chlorinated solvents. One field study demonstrating this technology was conducted at the Moffett Field Naval Air Station, Mountain View, California (Semprini et al., 1991). This site was used earlier to study in-situ restoration of chlorinated aliphatics by methanotrophic bacteria (Roberts et al., 1990). Reducing conditions were promoted in the field in a 2 square meter test zone by stimulating a consortium of denitrifying bacteria, and perhaps sulfate reducing bacteria, through the addition of acetate as primary substrate (25 mg/L). The aquifer contained both nitrate (25 mg/L) and sulfate (700 mg/L). CT was continuously injected at a concentration of 40 µg/L, and between 95 and 97 percent CT biotransformation was observed in the 2-m test zone with stimulated anaerobic growth. CF was an intermediate product and represented 30 to 60 percent of the CT transformed. Other halogenated aliphatics were biotransformed but at slower rates and lower extents of removal. Removals achieved for Freon-11, Freon-113, and 1,1,1-TCA ranged between 65 to 75 percent, 10 to 30 percent, and 11 to 19 percent, respectively.

A second field demonstration was conducted at a chemical transfer facility in North Toronto (Major et al., 1991). The aquifer at this site was contaminated with organic solvents (methanol, methyl ethyl ketone, vinyl and ethyl acetate, and butyl acrylate) and PCE. Samples of the aquifer material were amended with PCE plus acetate/methanol. Over a 145-day incubation period in the laboratory, PCE was dechlorinated to TCE, then *cis*-1,2-DCE, VC, and in many instances, to ethene. From these results the investigators hypothesized that the methanol present in the contaminated site serves as a primary substrate for complete dechlorination of PCE by anaerobic microorganisms. In-situ anaerobic bioremediation appears to be occurring at the site without addition of chemicals.

The possible formation of toxic metabolites has been the major impediment to the development of practical anaerobic bioremediation in the field for cleanup of chlorinated solvent contamination. The intermediates commonly observed, such as *cis*-1,2-DCE, *trans*-1,2-DCE, VC, 1,1-DCA, and CF, also pose a threat to public health. For anaerobic bioremediation to be useful, chlorinated solvents must be biotransformed to nonchlorinated, environmentally acceptable products. Some recent laboratory studies have demonstrated that this is possible and help provide impetus to further develop anaerobic biological processes for bioremediation.

7.3 Air Sparging

Air sparging is a potential means of extending the advantages of vapor extraction technology and bioventing to the saturated regime. With air sparging, air is injected under pressure below the water table creating a transient air-filled porosity. This enhances biodegradation as well as volatilization of petroleum hydrocarbon contaminants from the soil and ground water. The net result is a rapid and significant decrease in contaminant levels.

There is very little information available concerning effectiveness of air sparging at full scale in the United States. Air sparging is an emerging technology for the treatment of ground water contaminated with volatile organic compounds. It is being used to increasingly greater extents to treat petroleum hydrocarbon contaminated ground-water aquifers, overcoming the limitation of SVE for treating saturated zone contaminants and improving the efficacy of bioremediation. The benefits and limitations of this technology are still being defined both in field application and research.

7.4 Bioventing

Bioventing has been performed and monitored at several field sites contaminated with middle distillate fuels, mainly JP-4 jet fuel (Dupont et al., 1991; Miller et al., 1991; van Eyk and Vreeken, 1991; Urlings et al., 1991). Several large scale studies have been conducted at United States Air Force bases which have contributed significantly to knowledge concerning optimum design and operations. Results from these field demonstrations indicate that bioventing may be a feasible option for in situ biodegradation of residual fuel contaminants not amenable to recovery by SVE alone. Methods to reduce vapor extraction rates and maximize vapor retention times in the soil are compatible with enhancing biodegradation reactions through moisture management. Use of bioventing should minimize volatilization, potentially eliminate the need for off-gas treatment, and maximize in situ utilization of oxygen. It is estimated that various forms of bioventing have been applied to more than 1,000 sites worldwide; however, little effort has been given to the optimization of these systems.

The effects of environmental variables on bioventing treatment rates are still not well understood. In-situ respirometry at additional sites with drastically different geologic conditions has further defined environmental limitations and site-specific factors that are pertinent to successful bioventing. However,

the relationship between respirometric data and actual bioventing treatment rates have not been clearly determined. Additional field respirometry and closely monitored field pilot bioventing studies at the same sites are needed to determine what types of contaminants can be successfully treated in situ by bioventing and what the environmental limitations are. Studies to date clearly show that many preconceptions regarding the factors that control bioventing rates can be wrong. For example, active respiration at a subarctic site at Eielson AFB near Fairbanks, Alaska, suggests that good rates of in-situ hydrocarbon degradation can occur at locations that are continually subjected to a cold environment. Failure to accelerate biodegradation rates by adding nitrogen fertilizer to biovented soils that contain low nitrogen levels indicates that nutrient addition at some sites may not be required. Also, fine-grained moist clayey soils have been readily aerated and showed aerobic respiration, indicating that bioventing is feasible at times in soils having low permeabilities. Other low permeability sites have not proven amenable to bioventing, and better procedures to evaluate sites are needed.

Vapor phase biodegradation occurs and can take place in situ. The question of how soil sorption and partitioning of volatile organic compounds into soil air affects biodegradation rates was addressed earlier by McCarty (1987). This question needs further attention as the movement of the vapor phase in soils is complex and dependent on changing soil environmental conditions. Bioventing rates need to be determined under varying vapor extraction rates since an important purpose for bioventing is to biodegrade the vapor within the soil profile. The minimal soil aeration levels that provide for high degradation rates must be determined under different soil conditions. Interaction of the vapor phase with soil particles and microorganisms in the uncontaminated soil profile needs further research in both the laboratory and in the field. The primary problems encountered with bioventing are:

- Accurate estimate of emissions—One of the key variables in bioventing cost and design is the need for offgas treatment. Regulators typically require an estimate of emission rate before permitting a facility without offgas treatment.

- Time required for remediation—Bioventing may require two or more years for remediation. At many sites this can be a problem.

- Determination of effectiveness on nonpetroleum hydrocarbons—Many sites are contaminated with a mixture of chemical wastes, and little is known of the effectiveness of bioventing on nonpetroleum hydrocarbons.

- Regulatory acceptance—With this technology, as with many emerging technologies, obtaining regulatory acceptance can be difficult.

SECTION 8
SUMMARY

In-situ bioremediation technologies have been developed in response to cost and technical factors associated with excavation and incineration of contaminated soils and aquifers. These technologies differ primarily in the mechanisms of delivering nutrients and electron acceptors to contaminated subsurface soils and aquifers. Currently these delivery systems include ground water recirculation, air-sparging below the water table, and venting of unsaturated (vadose zone) soils. The primary limitations of in situ bioremediation technology are site geology and hydrogeology, which limit delivery rates and capacities. Therefore, comprehensive site characterization is vital to successful design and implementation of in-situ bioremediation systems. Without detailed information concerning site hydrogeology, and nature and distribution of contaminants, appropriate designs cannot be selected. Performance evaluation for bioremediation also depends on adequate site characterization.

Although a wide range of contaminants are potentially biodegradable, bioremediation systems have been most successfully applied to petroleum hydrocarbons (fuels and refinery wastes), wood preserving wastes (creosote), and chlorinated solvents (TCE). Petroleum hydrocarbons and wood-preserving wastes are primarily biodegraded under aerobic conditions, using oxygen as an electron acceptor. Bioremediation of these contaminants has focused on mechanisms for overcoming oxygen limitations. Some petroleum hydrocarbons, principally aromatic components of fuels (BTEX), and chlorinated solvents (PCE, TCE) have been shown to biodegrade under anaerobic conditions using electron acceptors other than oxygen. Nitrate, sulfate, and carbon dioxide are attractive alternatives to oxygen because they are more soluble in water, inexpensive, and nontoxic to microorganisms. The demonstration of this technology in the field is limited, therefore, its use for bioremediation must be viewed as a developing treatment technology.

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