



Project Summary

In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies

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Bioremediation of excavated soil, unsaturated soil, or ground water involves the use of microorganisms to convert contaminants to less harmful species in order to remediate contaminated sites. Bioremediation sometimes offers significant advantages over other types of remediation technologies. This report provides the most recent scientific understanding of the processes involved with soil and ground-water bioremediation and discusses the applications and limitations of the various in-situ bioremediation technologies. In order for the information in this document to be of maximum benefit, it is important that the reader understand how contaminants are distributed among the various subsurface compartments. This distribution, or phase partitioning of contaminants, is dependent upon a number of factors including the characterization of the contaminants themselves and that of the subsurface environment. This distribution is exemplified in Figure 1 where contaminants are shown to be associated with the vapor phase in the unsaturated zone, a residual phase, or dissolved in ground water.

This Project Summary was developed by EPA's Robert S. Kerr Environmental Research Laboratory, Ada, OK, to announce key findings of the research project that is fully documented in a separate report of the same title (see

Project Report ordering information at the back).

Introduction

In-situ bioremediation of subsurface environments involves the use of microorganisms to convert contaminants to less harmful products and sometimes offers significant potential advantages over other remediation technologies. In order for these biodegradative processes to occur, microorganisms require the presence of certain minerals, referred to as nutrients, and an electron acceptor. Several other conditions, i.e., temperature and pH, impact the effectiveness of these processes. The use of biooxidation for environmental purposes has existed for many years and has led to considerable information regarding the biodegradability of specific classes of compounds, nutrient and electron acceptor requirements, and degradation mechanisms. Activated sludge and other suspended growth systems have been used for decades to treat industrial and municipal wastes. Land treatment processes for municipal wastewater and petroleum refinery and municipal wastewater sludges have also been practiced for several decades and have generated a great deal of information on nutrient requirements, degradation rates, and other critical parameters affecting biological oxidation.



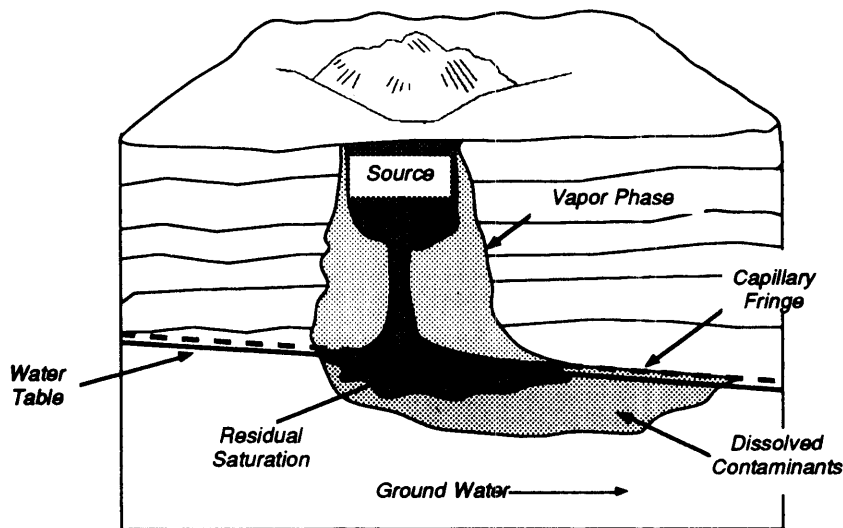


Figure 1. Distribution of contaminants in the subsurface.

In the 1970s, tests were conducted to evaluate biological degradation of petroleum hydrocarbons in aquifers. Results from these tests demonstrated that in-situ bioremediation could reduce levels of hydrocarbons in aquifers and provided considerable information concerning the processes which take place and the requirements necessary to drive these processes.

Although a variety of minerals are required by the microorganisms, it is usually necessary to add only nitrogen and phosphorus. The most common electron acceptor used in bioremediation is oxygen. Stoichiometrically, approximately three pounds of oxygen are required to convert one pound of hydrocarbon to carbon dioxide. Nutrient requirements are less easily predicted. If all hydrocarbons are converted to cell material, however, it can be assumed that nutrient requirements of carbon to nitrogen to phosphorus ratios are in the order of 100:10:1. In some cases where the levels of contaminants are low, sufficient nitrogen and phosphorus are naturally present, and only oxygen is required for the biological processes to proceed.

In-situ bioremediation systems for aquifers typically consist of extraction points such as wells or trenches, and injection wells or infiltration galleries. In most cases, the extracted ground water is treated prior to the addition of oxygen and nutrients, followed by subsequent reinjection.

Critical to the design of an in-situ bioremediation system is the ground-water flow

rate and flow path. The ground-water flow must be sufficient to deliver the required nutrients and oxygen 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 that limits the effectiveness of in-situ technologies or prevents its utility entirely. The results of a number of referenced 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.

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.

As a class, petroleum hydrocarbons are biodegradable. The lighter soluble members are generally biodegraded more rap-

idly and to lower residual levels than are the heavier, less soluble members. 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.

Polyaromatic hydrocarbons are present in heavier petroleum hydrocarbon blends and particularly in coal tars, wood treating chemicals, and refinery waste sludges. These compounds have only limited solubility in water, adsorb strongly to soils, and degrade at rates much slower than monoaromatic hydrocarbons.

Nonchlorinated solvents used in a variety of industries are generally biodegradable. For example, alcohols, ketones, ethers, carboxylic acids, and esters are readily biodegradable but may be toxic to the indigenous microflora at high concentrations due to their high water solubility.

Lightly chlorinated compounds such as chlorobenzene, dichlorobenzene, chlorinated phenols, and the lightly chlorinated PCBs are typically biodegradable under aerobic conditions. The more highly chlorinated analogs are more recalcitrant to aerobic degradation but more susceptible to degradation under anaerobic conditions.

Chlorinated solvents and their natural transformation products represent the most prevalent organic ground-water contaminants. These solvents, consisting primarily of chlorinated aliphatic hydrocarbons, have been widely used for degreasing aircraft engines, automotive parts, electronic components, and clothing.

In-situ biodegradation of most of these solvents depends upon cometabolism and can be carried out under aerobic or anaerobic conditions. Cometabolism requires the addition of an appropriate primary substrate to the aquifer and perhaps an electron acceptor, such as oxygen or nitrate, for its oxidation.

In the early 1980s there were few companies that had experience in the bioremediation of soil and ground water. Since that time, many companies have used bioremediation technologies, although claims of experience are frequently overstated. There now exists a number of organizations and specialists that are knowledgeable in the field of in-situ bioremediation. Several environmental companies have staffs that are experienced in the application of this technology. Many large corporations, especially the oil and chemical companies, have also developed in-house expertise. Some of the

U.S. Environmental Protection Agency laboratories, as well as Department of Defense and Department of Energy groups, have conducted laboratory research and field demonstration studies concerning bioremediation.

Bioventing

Bioventing is the process of supplying air or oxygen to soil to stimulate the aerobic biodegradation of contaminants. This technology is applicable to contaminants in the vadose zone and contaminated regions of an aquifer just below the water table. This in-situ process may be applied to the vadose zone as well as an extended unsaturated zone caused by dewatering. Bioventing is a modification of the technology referred to as soil vacuum extraction, vacuum extraction, soil gas extraction, and in-situ volatilization.

Laboratory research and field demonstrations involving bioventing began in the early 1980s, with particular emphasis on the remediation of soil contaminated with hydrocarbons. 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. Much of the success of this technology is because the use of air as a carrier of oxygen is 1,000 times more efficient than water. 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.

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. 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.

Air Sparging

Air sparging is the injection of air under pressure below the water table to create a transient air-filled porosity by displacing water in the soil matrix. Air sparging is a remediation technology applicable to contaminated aquifer solids and vadose zone materials. 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 but was not introduced to the United States until recently.

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.

One of the problems in applying air sparging is controlling the process. In either bioventing or ground-water extraction, the systems are under control because contaminants are drawn to the point of collection. By contrast, air sparging systems cause water and contaminants to move away from the point of injection which can accelerate and aggravate the spread of contamination. Changes in lithology can profoundly affect both the direction and velocity of air flow. A second problem in air sparging is accelerated vapor travel. Since air sparging increases the vapor pressure in the vadose zone any exhausted vapors could be drawn into receptors such as basements. As a result, in areas with potential vapor receptors, air sparging should be done with vent systems which allow an effective means of capturing sparged gases.

As with any technology, there are limitations to the utility and applicability of air sparging. The first is associated with the type of contaminants to be removed. For air sparging to work effectively, the contaminant must be relatively volatile and relatively insoluble. If the contaminant is soluble and nonvolatile, it must be biodegradable. The second limitation to the use of air sparging is the geological character of the site. The most important geological characteristic is the homogeneity of the site. If significant stratification is present, there is a danger that sparged air could be held below an impervious layer and spread laterally, thereby resulting in the spread of contamination.

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 for a sufficient thickness 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.

Alternate Electron Acceptors

Bioremediation using electron acceptors other than oxygen is potentially advantageous for overcoming the difficulty in supplying oxygen for aerobic processes. 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 as an alternate electron acceptor for bioremediation must be viewed as a developing treatment technology.

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

Bioremediation with alternate electron acceptors involves the stimulation of microbial growth by the perfusion of electron donors, electron acceptors, and nutrients through the formation. Addition of alternate electron acceptors other than nitrate for bioremediation has not been documented at field scale but has been widely studied at laboratory scale. Nitrate as an electron acceptor has been used for bioremediation of benzene, toluene, ethylbenzene, and xylenes in ground water and on aquifer solids. As for other in-situ remediation technologies, formations with hydraulic conductivities of 10^{-4} cm/sec (100 ft/sec) or greater are most amenable to bioremediation.

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, and susceptibility to reduction increases with the number of chlorine substitutes. Conversely, 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 a potential utility as a control technology for chlorinated solvent contamination.

Natural Bioremediation

The basic concept behind natural bioremediation is to allow naturally occurring microorganisms to degrade contaminants that have been released into the subsurface. It is not a "no action" alternative, as in most cases it is used to supplement

other remediation techniques. In some cases, only the removal of the primary source may be necessary. In others, conventional ground-water remediation techniques such as pump-and-treat may be used to reduce contaminant concentrations within the aquifer.

Natural bioremediation is capable of treating contaminants aerobically in the vadose zone and at the margins of plumes, where oxygen is not limiting. 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.

While there are no "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 2). 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 hydrocarbons may biodegrade. The extent of the biodegradation will be controlled by the size of the spill, the rate of downward movement, and the appropriateness of requisite environmental conditions. Dissolved hydrocarbons that are not completely de-

graded 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 and 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.

One of the major factors controlling the use of natural bioremediation is the acceptance of this approach by regulators, environmental groups, and the public. The implementation of these systems differs from conventional techniques in that a portion of the aquifer is allowed to remain contaminated. This results in the necessity of obtaining variances from regulations, and some type of risk evaluation is usually required. Even when public health is not at risk, adjoining land owners may have strong concerns about a contaminant plume migrating under and potentially impacting their property. Therefore, control of plume migration at these sites, usually using some type of hydraulic system, is often necessary. Although natural bioremediation imposes few costs other than monitoring and the time for natural processes to proceed, the public may perceive that this is a "no action" alternative. These various factors may generate opposition to selecting natural bioremediation rather than conventional technologies.

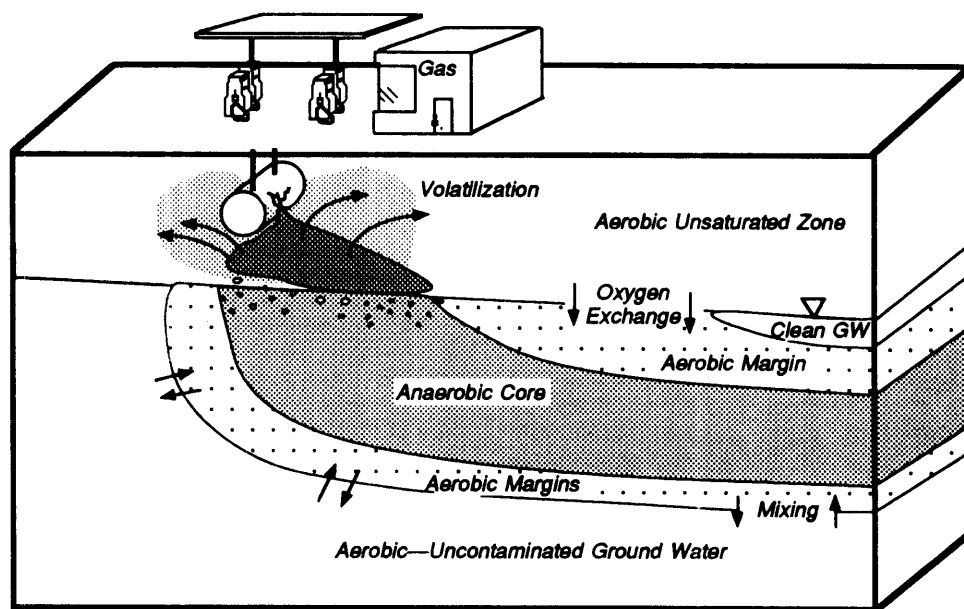


Figure 2. Profile of a hypothetical hydrocarbon plume undergoing passive bioremediation.

There is almost no operating history to judge the effectiveness of natural bioremediation. In addition, there are currently no reliable methods for predicting its effectiveness without first conducting extensive field testing. This is often the primary reason why natural bioremediation is not seriously considered when evaluating remedial alternatives. At many low priority sites, regulators may have assumed that natural bioremediation would control the migration of dissolved contaminants. Often, these sites have not been adequately characterized nor have they been monitored to determine the effectiveness of this remediation technology. At present, there are no well-documented, full-scale investigations of natural bioremediation. Such investigations will be necessary to define site and contaminant characteristics conducive to this remediation alternative before it can be accepted with confidence by practitioners, regulators, and the public.

Introduced Organisms

Historically, the movement of microorganisms in the subsurface was first discussed in the mid-1920s in relation to the enhanced recovery of oil by the production of biological surfactants and gases. Later, the transport of bacteria through soil was studied to measure the effectiveness of soil-based sewage treatment fa-

cilities such as pit latrines and septic tanks in terms of the removal of pathogens. 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. In these attempts to introduce microorganisms to the subsurface, it is often difficult to differentiate their activities from indigenous populations. The use of introduced microorganisms has proven most successful in surface bioreactors when treating extracted ground water in closed-loop recirculation systems.

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 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.

Summary

This report has been prepared by leading soil and ground-water remediation scientists in order to present the latest technical, institutional, and cost considerations applicable to subsurface remediation systems. It is aimed at scientists, consultants, regulatory officials, and others who are, in various ways, working to achieve efficient and cost-effective remediation of contaminants in the subsurface environment.

The document contains detailed information about the processes, applications, and limitations of using remediation technologies to restore contaminated soil and ground water. Field tested as well as new and innovative technologies are discussed. In addition, site characterizations requirements for each remediation technology are discussed along with the costs associated with their implementation. A number of case histories are presented, and knowledge gaps are pointed out in order to suggest areas for which additional research investigations are needed.

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John E. Matthews is the EPA Project Officer (see below).

The complete report, entitled "In-Situ Bioremediation of Ground Water and Geological Material: A Review of Technologies," (Order No. PB93-215564; Cost \$36.50) will be available only from:

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