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Engineering Issue

In Situ and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites

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1.0 PURPOSE

The U.S. Environmental Protection Agency (EPA) Engineering Issues are a new series of technology transfer documents that summarize the latest available information on selected treatment and site remediation technologies and related issues. They are designed to help remedial project managers (RPMs), on-scene coordinators (OSCs), contractors, and other site managers understand the type of data and site characteristics needed to evaluate a technology for potential applicability to their specific sites. Each Engineering Issue document is developed in conjunction with a small group of scientists inside the EPA and with outside consultants and relies on peer-reviewed literature, EPA reports, Internet sources, current research, and other pertinent information. For this Engineering Issue paper, the reader is assumed to have a basic technical background and some familiarity with bioremediation. Those readers interested in a more basic discussion of bioremediation should consult the A *Citizen's Guide to Bioremediation* (EPA, 2001a).

The purpose of this Engineering Issue paper for biodegradation technologies is to summarize current information on bioremediation and to convey that information clearly and concisely to site managers. The Table of Contents indicates the types of information covered in this Engineering Issue paper, and this information relies, wherever feasible, on independently reviewed process performance information. In an effort to keep this Engineering Issue paper short, important information is summarized, while references and Internet links are provided for readers interested in additional information; these Internet links, verified as accurate at the time of publication, are subject to change.

2.0 INTRODUCTION

Bioremediation is a grouping of technologies that use microbiota (typically, heterotrophic bacteria and fungi) to degrade or transform hazardous contaminants to materials such as carbon dioxide, water, inorganic salts, microbial biomass, and other byproducts that may be less hazardous than the parent materials. Biological treatment has been a major component for many years in the treatment of municipal and industrial wastewaters. In recent years, biological mechanisms have been exploited to remediate contaminated ground water and soils (EPA, 1998a; EPA, 2000). This Engineering Issue paper focuses on bioremediation technologies for treating contaminated soils, sediments, sludges, ground water, and surface water since these are

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the matrices typically found at contaminated sites. Treatments for municipal wastewater, industrial wastewater, and storm water will not be discussed.

During the late 1970s, 1980s, and early 1990s, wastewater treatment[1] and composting technologies were adapted to new applications. At that time, bioremediation of any hazardous constituents or waste was considered innovative. However, numerous applications of bioremediation are now widely accepted as a remedial alternative and are in wide use at sites contaminated with petroleum products and/or hazardous wastes. Some bioremediation technologies, such as cometabolic bioventing, are still in development and should be considered innovative. Other bioremediation technologies, such as anaerobic bioventing, are current topics of research. Since bioremediation technologies that fall into the innovative or research category have limited field implementation and effectiveness data, additional site assessment and treatability studies may be needed to confirm that a selected technology will be effective at a specific site.

According to the EPA Office of Solid Waste and Emergency Response annual treatment technology status report (10th Edition) covering the period of Fiscal Year (FY) 1982 through FY 1999, bioremediation was planned or implemented for source control and ground water remediation for 105 Superfund Remedial Action and 51 Superfund Removal Action projects. In some cases, bioremediation was applied at multiple operable units on a site, each of which is included as a project (EPA, 2001b). The following contaminants have been bioremediated successfully at many sites:

- Halogenated and non-halogenated volatile organic compounds (VOCs)
- Halogenated and non-halogenated semi-volatile organic compounds (SVOCs).

Contaminants with a more limited bioremediation performance include:

- Polycyclic aromatic hydrocarbons (PAHs)
- Organic pesticides and herbicides
- Polychlorinated biphenyls (PCBs).

Although the applications of bioremediation under the EPA Superfund Program, as discussed above, involve some of the most difficult sites and well-documented site surveys, these examples represent only a fraction of the bioremediation applications nationwide and worldwide. Larger numbers of sites are handled under the Resource Conservation and Recovery Act (RCRA), state-led remediation programs, leaking underground storage tank (LUST) programs, and state voluntary cleanup programs. For example, a survey in 2001 showed that biodegradation technologies, such as land farming and biopiles, are applied at 33% of the soil LUST sites. The same survey showed that *in situ* bioremediation, biosparging, and technologies that may use bioremediation, such as monitored natural attenuation, are used on 79% of the ground water plumes at LUST sites (Kostecki and Nascarella, 2003). However, applications of various bioremediation technologies at other site types are not summarized, so their use is difficult to characterize.

Bioremediation remains an active field of technology research and development at both the laboratory and field scale. For example, applications to chlorinated aliphatic hydrocarbons (CAHs), perchlorate, and methyl tert-butyl ether (MTBE) were developed rapidly in recent years. Contaminants with a more limited bioremediation performance record include:

- Applications to additional contaminants and matrices, such as (but not limited to) trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), pesticides and herbicides, and dense non-aqueous phase liquids (DNAPLs)
- Delivery of treatment in difficult media (i.e., fractured bedrock or tight clays)
- Refinement of strategies for cost-effective system design and operation.

The field of bioremediation can be divided into several broad categories. For example, bioremediation technologies may be applied to *in situ* or *ex situ* media. *In situ processes* treat soils and ground water in place, without removal. This approach may be advantageous since the costs of materials handling and some environmental impacts may be reduced. However, *in situ* processes may be limited by the ability to control or manipulate the physical and chemical environment in place. An example of an *in situ* bioremediation technology is aerobic bioventing, which has been used at many sites to treat subsurface soils contaminated by fuels. In aerobic bioventing, air is typically injected into the subsurface to facilitate aerobic metabolism of hydrocarbons. *Ex situ processes* involve the removal of the contaminated media to a treatment area.

[1] Historical information on the development of bioremediation can be found in Martin and Gershuny, 1992; Section 2.3 of EPA, 1993a; and Bradley, 2003.

Examples of *ex situ* processes include land treatment and composting. In these processes, soils are excavated, mixed with amendments, and operated in a manner that facilitates degradation of the contaminants of concern.

Another way to divide the bioremediation field is based on additives to environmental media. *Intrinsic bioremediation* depends on indigenous microflora to degrade contaminants without any amendments (EPA, 2000). This approach is used *in situ* and takes advantage of pre-existing processes to degrade hazardous wastes. Intrinsic bioremediation requires careful site assessment and monitoring to make sure that the ongoing processes are protective of environmental receptors. Temperature, pH, and other factors may also be adjusted and monitored to enhance bioremediation. Alternatively, *enhanced bioremediation* facilitates biodegradation by manipulating the microbial environment, typically by supplying chemical amendments such as air, organic substrates or electron donors, nutrients, and other compounds that affect metabolic reactions (EPA, 2000). Enhanced bioremediation may also be called *biostimulation* when only chemical amendments are added. Examples of biostimulation include bioventing, land farming or land treatment, biopiles, composting, and sometimes anaerobic reductive dechlorination. Biostimulation technologies may be applied to *in situ* or *ex situ* situations and may be used to treat soil and other solids, ground water, or surface water. In some cases, *bioaugmentation*, which involves the addition of microbial cultures, is used to enhance biotreatment. Bioaugmentation may be needed for specific contaminants that are not degraded by the indigenous organisms. Bioaugmentation is almost always performed in conjunction with biostimulation. For example, bioaugmentation has been used at some chlorinated solvent sites as a modification of anaerobic reductive dechlorination when indigenous microbes were unable to completely dechlorinate the contaminants of concern.

In bioremediation, fundamental biological activities are exploited to degrade or transform contaminants of concern. The biological activity to be exploited depends on the specific contaminants of concern and the media where the contamination is located. For example, in *aerobic* environments, many microbes are able to degrade organic compounds, such as hydrocarbons. These microbes gain energy and carbon for building cell materials from these biochemical reactions. At many sites with fuel contamination, the amount of oxygen present limits the extent of biotreatment. Thus, by adding oxygen in the form of air, contaminant degradation proceeds directly.

In *cometabolism*, microbes do not gain energy or carbon from degrading a contaminant. Instead, the contaminant is degraded via a side reaction. Technologies based on cometabolism are more difficult to use since the microbes do not benefit from the desired reactions. Cometabolic bioventing is an example of cometabolism. In this technology, microbes may be fed propane, and they degrade trichloroethylene (TCE) or less chlorinated ethenes as well.

Depending on the contaminant of concern and the media, a technology may exploit aerobic or anaerobic metabolism. Aerobic metabolism is more commonly exploited and can be effective for hydrocarbons and other organic compounds. Many organisms are capable of degrading hydrocarbons using oxygen as the electron acceptor and the hydrocarbons as carbon and energy sources. In some cases, contaminants are aerobically degraded to carbon dioxide, water, and microbial biomass, but in other cases, the microbes do not completely degrade contaminants. Aerobic technologies may also change the ionic form of metals. If a site contains mixed metal and organic wastes, it is necessary to consider whether the oxidized forms of the metal species (such as arsenic) will be environmentally acceptable.

Anaerobic metabolism involves microbial reactions occurring in the absence of oxygen and encompasses many processes including fermentation, methanogenesis, reductive dechlorination, sulfate-reducing activities, and denitrification. Depending on the contaminant of concern, a subset of these activities may be cultivated. In anaerobic metabolism, nitrate, sulfate, carbon dioxide, oxidized metals, or organic compounds may replace oxygen as the electron acceptor. For example, in anaerobic reductive dechlorination, chlorinated solvents may serve as the electron acceptor.

Phytoremediation or phytotechnology, which involves the use of plants to remediate contaminated media, is not discussed in this Engineering Issue paper because this technique can involve a number of physical and chemical processes in addition to, or in place of, bioremediation. More information on phytoremediation can be found at the EPA Web site (http://www.clu-in.org/techfocus/default.focus/sec/Phytoremediation/cat/Overview/) or at the Interstate Technology & Regulatory Council (ITRC) Web site (http://www.itrcweb.org/gd_Phyto.asp).

When selecting a bioremediation technology, it is important to consider the contaminants of concern, contaminated matrix, potential biological pathways to degrade a contaminant, and current conditions at a site. For ex-

ample, TCE can be degraded via aerobic and anaerobic mechanisms. If ground water is contaminated with TCE, current ground water conditions may be helpful in deciding which biological mechanism to exploit. If ground water is already anaerobic, then anaerobic reductive dechlorination may be the best approach. However, if the TCE plume is diffuse and the ground water is aerobic, it may be possible to use cometabolic technologies.

A key concept in evaluating all bioremediation technologies is microbial bioavailability. Simply stated, if the contaminant of concern is so tightly bound up in the solid matrix (either chemically or physically) that microorganisms cannot access it, then it cannot be bioremediated. However, low microbial bioavailability does not imply an absence of risk; compounds may be available to environmental receptors depending on the receptor and routes of exposure. For example, if a child ingests contaminated soil, digestive processes may release contaminants that were not available to microbes.

Many of the guidance documents discussed in this Engineering Issue paper address bioavailability from a practical engineering perspective. A more theoretical summary of the implications of bioavailability is presented in Luthy et al. (1997) and Alexander (2000). Implementation of enhanced bioremediation technologies can, in some cases, transfer mass from the solid phase to the mobile (i.e., water) phase through a number of mechanisms, including the generation of biosurfactants.

Thus, when selecting a bioremediation technology for a specific site, it is prudent to consider the contaminants of concern, potential degradation intermediates and residuals of the contaminants, co-contaminants, environmental receptors, routes of exposure, and buffer zones between contamination and receptors. Bioremediation technologies have proven to be protective and cost-effective solutions at many sites. However, conditions at a specific site may not be appropriate. In addition, worker safety issues are a consideration in selection, design, and operation of bioremediation technologies. These technologies may involve the use of strong oxidants and/or highly reactive, potentially explosive chemicals.

3.0 TECHNOLOGY DESCRIPTION

This section discusses *in situ* and *ex situ* bioremediation. Technologies within each of these broad categories are presented, including representative process schematics or illustrations. Note that there are multiple vendors of some technologies, each applying proprietary components or processes to their particular technology. The continually changing nature of bioremediation and the space limitations of this Engineering Issue paper preclude comprehensive presentation of such information. Additional information on remediation technologies and links to other sources are available on the World Wide Web, including:

- Federal Remediation Technologies Roundtable (FRTR) at http://www.frtr.gov/
- Air Force Center for Environmental Excellence (AFCEE) Technology Transfer Program at http://www.afcee.brooks.af.mil/products/techtrans/treatmenttechnologies.asp
- ITRC guidance documents and case studies at http://www.itrcweb.org/gd.asp and http://www.itrcweb.org/successstories.asp.

For information on specific compounds, a Biocatalysis/Biodegradation Database developed by the University of Minnesota may be helpful and can be found at http://umbbd.ahc.umn.edu/.

3.1 In Situ Bioremediation

There are two major types of *in situ* bioremediation: intrinsic and enhanced. Both rely on natural processes to degrade contaminants with (enhanced) or without (intrinsic) amendments.

In recent years, *in situ* bioremediation concepts have been applied in treating contaminated soil and ground water. Removal rates and extent vary based on the contaminant of concern and site-specific characteristics. Removal rates also are affected by variables such as contaminant distribution and concentration; co-contaminant concentrations; indigenous microbial populations and reaction kinetics; and parameters such as pH, moisture content, nutrient supply, and temperature. Many of these factors are a function of the site and the indigenous microbial community and, thus, are difficult to manipulate. Specific technologies may have the capacity to manipulate some variables and may be affected by other variables as well; these specific issues are discussed with each technology in the following sections (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003).

When *in situ* bioremediation is selected as a treatment, site-monitoring activities should demonstrate that biologically mediated removal is the primary route of contaminant removal. Sampling strategies should consider appropriate analytes and tests, as well as site heterogeneity. In some

cases, extensive sampling may be required to distinguish bioremediation from other removal mechanisms or statistical variation. Small-scale treatability studies using samples from the contaminated site may also be useful in demonstrating the role that biological activity plays in contaminant removal (EPA, 1995b; EPA, 1998a; EPA, 2000).

3.1.1 Intrinsic *In Situ* Bioremediation

Intrinsic bioremediation relies on natural processes to degrade contaminants without altering current conditions or adding amendments. Intrinsic bioremediation may play a role in monitored natural attenuation (MNA) sites, which is a broader term defined by the National Research Council (NRC) and EPA as *"biodegradation, dispersion, dilution, sorption, volatilization, radioactive decay, and chemical or biological stabilization, transformation or destruction of contaminants"* (NRC, 2000; EPA, 1999).

Natural attenuation (NA) relies on natural physical, chemical, and biological processes to reduce or attenuate contaminant concentrations. Under favorable conditions, NA will reduce the concentration, mass, toxicity, mobility, and/or volume of contaminants in soil and ground water. Natural processes involved in NA include dilution, dispersion, sorption, volatilization, chemical reactions such as *oxidation* and reduction, biological reactions, and stabilization. EPA prefers those processes that degrade contaminants and expects that NA will be most appropriate for subsurface plumes that are stable. Some processes have undesirable results, such as creation of toxic degradation products or transfer of contaminants to other media as noted in the *Seminar Series on Monitored Natural Attenuation for Ground Water* (EPA, 1998a).

Implementing MNA requires a thorough site assessment and development of a conceptual model of the site. After determining the presence of a stable or shrinking plume, site-specific, risk-based decisions using multiple lines of evidence may facilitate implementation of MNA at a site. While MNA is somewhat passive in that nothing is being added to the contamination zone, MNA requires active monitoring, which should be included as part of the design plan for a site. In some cases, such long-term monitoring may be more expensive than active remediation. MNA is only applicable to carefully controlled and monitored sites and must reduce contaminant concentrations to levels that are protective of human health and the environment in reasonable timeframes (EPA, 1998a). Depending on site-specific conditions, MNA may be a reasonable alternative for petroleum hydrocarbons as well as chlorinated and non-chlorinated VOCs and SVOCs (EPA, 1999a; EPA, 1999b).

Good overviews of natural attenuation are provided by:

- Natural Attenuation for Groundwater Remediation (NRC, 2000)
- The MNA page of the AFCEE Technology Transfer Program Web site at http://www.afcee.brooks.af.mil/products/techtrans/monitorednaturalattenuation/default.asp.
- ITRC *in situ* bioremediation publications at http://www.itrcweb.org/gd_ISB.asp.

Detailed protocols for evaluation of natural attenuation at sites with petroleum hydrocarbon and CAH contamination can be found at http://www.afcee.brooks.af.mil/products/techtrans/monitorednaturalattenuation/Protocols.asp.

3.1.2 Enhanced *In Situ* Bioremediation

Enhanced bioremediation can be applied to ground water, vadose zone soils, or, more rarely, aquatic sediments. Additives such as oxygen (or other electron acceptors), nutrients, biodegradable carbonaceous substrates, bulking agents, and/or moisture are added to enhance the activity of naturally occurring or indigenous microbial populations (FRTR, 2003).

3.1.2.1 Vadose Zone Soil Remediation

While the fundamental biological activities exploited by *in situ* bioremediation may occur naturally, many sites will require intervention to facilitate cleanup. For example, the addition of organic substrates, nutrients, or air may provide the appropriate environment for specific microbial activities or enhanced removal rates. In general, hydrocarbons and lightly chlorinated contaminants may be removed through aerobic treatment. Highly chlorinated species are degraded primarily through anaerobic treatment. Both anaerobic and aerobic treatment may occur through direct or cometabolic pathways (EPA, 2000).

The primary *in situ* biological technology applicable to the unsaturated zone is bioventing, which is categorized as either aerobic, cometabolic, or anaerobic depending on the amendments used.

3.1.2.1.1 Aerobic Bioventing

Bioventing has a robust track record in treating aerobically degradable contaminants, such as fuels. In aerobic bioventing, contaminated unsaturated soils with low oxygen concentrations are treated by supplying oxygen to facilitate aerobic microbial biodegradation. Oxygen is typically introduced by air injection wells that push air into the subsurface (see Figure 3-1); vacuum extraction wells, which draw air through the subsurface, may also be used. When building foundations or similar structures are close to the site, the extraction mode may be used to avoid the buildup of contaminated, and possibly explosive, vapors in the building basements. Extracted gases may require treatment since volatile compounds may be removed from the ground. Compared with soil vapor extraction (SVE), bioventing employs lower air flow rates that provide only the amount of oxygen required to enhance removal. Operated properly, the injection of air does not result in the release of the contaminants to the atmosphere through volatilization because of these low flow rates (AFCEE, 1996; EPA, 2000; FRTR, 2003).

Bioventing is designed primarily to treat aerobically biodegradable contaminants, such as non-chlorinated VOCs and SVOCs (e.g., petroleum hydrocarbons), that are located in the vadose zone or capillary fringe (EPA, 2000; FRTR, 2003). The U.S. Air Force Bioventing Initiative and the EPA Bioremediation Field Initiative demonstrated that bioventing was effective under a wide variety of site conditions at about 125 sites. The experience from bioventing demonstrations at these sites was condensed into a manual, *Bioventing Principles and Practice* (EPA 1995a), which provides information about the applicability of bioventing and outlines its use and design (AFCEE, 1996). Data collected during the bioventing demonstrations also provide information about the rates of contaminant removal observed. In addition to the variables discussed initially, bioventing rates and system design are affected by soil gas permeability, soil water content, depth to contamination, and oxygen supply and radius of influence (EPA, 2000; FRTR, 2003). The costs for aerobic bioventing are about $50/cubic yard (AFCEE, 1996).

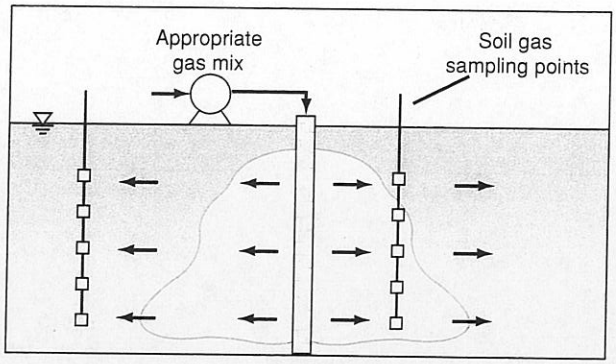

Figure 3-1. Aerobic bioventing in injection mode. (Adapted from EPA, 2004c)

Based on experience gained to date in applying bioventing to fuels, site heterogeneity is a principal impediment to establishing that biological activity is the principal mode of removal at these sites. Measurements of the rate and amount of contaminant removed, oxygen supply, and carbon dioxide generation, as well as mass balances relating to these three amounts, may be useful in establishing bioremediation as the primary mechanism of removal. For sites where other contaminants are to be treated by bioventing, other factors may be considered in establishing biological activity as the primary mechanism of removal (AFCEE, 1996; EPA, 2000; FRTR, 2003).

Regulatory acceptance of this technology has been obtained in 30 states and in all 10 EPA regions (FRTR, 2003). The use of this technology in the private sector is increasing following the U.S. Air Force Bioventing Initiative and the EPA Bioremediation Field Initiative.

In addition to fuels treatment, aerobic bioventing has treated a variety of other contaminants including non-halogenated solvents such as benzene, acetone, toluene, and phenol; lightly halogenated solvents such as 1,2-dichloroethane, dichloromethane, and chlorobenzene; and SVOCs such as low-molecular-weight PAHs. Since the experience with these other types of contaminants is more limited, laboratory- and pilot-scale studies may be needed to evaluate effectiveness, design the bioventing system, and estimate treatment times.

Bioventing has proven to be a useful technology at many sites under a variety of conditions, but like all technologies, bioventing has some limitations. One set of bioventing limitations involves the ability to deliver oxygen to the contaminated soil. For example, soils with extremely high moisture content may be difficult to biovent because of reduced soil gas permeability. Similarly, low-permeability soils also may pose some difficulties for bioventing because of a limited ability to distribute air through the subsurface. In both cases, the design of the bioventing system may be able to compensate for low permeability. Additionally, sites with shallow contamination can pose a challenge to bioventing because of the difficulty in developing a system design that can minimize environmental release and achieve sufficient aeration. In this situation,

operating the system in the extraction mode may circumvent the difficulty (AFCEE, 1996; FRTR, 2003).

Another limitation is that bioventing will not stimulate contaminant bioremediation if the contaminated zone is aerobic. If a soil gas survey measures soil oxygen levels consistently above 2–5%, then the soil is sufficiently aerated for biodegradation to occur and oxygen is not limiting degradation. Bioventing will not enhance bioremediation in this situation. This situation is unusual and, if encountered, may indicate that some other contaminants, such as metals, are inhibiting degradation (AFCEE, 1996; EPA, 1998a).

While bioventing is relatively inexpensive, bioventing can take a few years to clean up a site depending on contaminant concentrations and site-specific removal rates. If a quicker cleanup is needed, more intensive *ex situ* technologies may be more appropriate (AFCEE, 1996; EPA, 2000; FRTR, 2003).

3.1.2.1.2 Cometabolic Bioventing

Cometabolic bioventing has been used at a few sites to treat chlorinated solvents such as TCE, trichloroethane (TCA), and dichloroethene (DCE). The equipment used in cometabolic bioventing is similar to aerobic bioventing, but cometabolic bioventing exploits a different biological mechanism. Similar to bioventing, cometabolic bioventing involves the injection of gases into the subsurface; however, cometabolic bioventing injects both air and a volatile organic substrate, such as propane. The concentrations in this gas mixture should be well below the lower explosive limit (LEL), and should be monitored in soil gas (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003).

Cometabolic bioventing exploits competitive reactions mediated by monooxygenase enzymes (EPA, 2000). Monooxygenases catalyze the oxidation of hydrocarbons, often through epoxide intermediates, but these enzymes can also catalyze the dechlorination of chlorinated hydrocarbons. Thus, by supplying an appropriate organic substrate and air, cometabolic bioventing can elicit the production of monooxygenases, which consume the organic substrate and facilitate contaminant degradation (AFCEE, 1996; EPA, 1998a).

Cometabolic bioventing has been used to treat lightly chlorinated compounds in the vadose zone or capillary fringe. In addition to the variables discussed in the previous aerobic bioventing section, the degradation rate and design of cometabolic bioventing systems are dependent on many factors including soil gas permeability, organic substrate concentration, type of organic substrate selected, and oxygen supply and radius of influence. Unlike many variables that are determined by site conditions, the selection and concentration of the organic substrate are controllable and can be important to the removal rate. Treatability or bench-scale testing can be useful in selecting the organic substrate and concentration for a site. In addition, small-scale testing can demonstrate that full dechlorination is observed at a site (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003).

Establishing cometabolic bioventing as the primary mechanism of removal in the field is challenging. Unlike aerobic bioventing, the oxygen use and chlorinated solvent removal are not related stoichiometrically because the metabolism of added organic substrates also consumes oxygen. As a result, measurements of oxygen use, carbon dioxide generation, and contaminant removal cannot be linked stoichiometrically. Indirect measures, such as measuring chloride ion accumulation in the soil and correlating that accumulation to contaminant removal, have been useful at some sites. In addition, collecting data to demonstrate degradation of the organic substrate (by a shutdown test) in the field may be helpful, especially in conjunction with laboratory testing using contaminated soil from the site (AFCEE, 1996; EPA, 1998a; FRTR, 2003).

Cometabolic bioventing has been successfully demonstrated at a few sites. The Remediation Technology Development Forum (RTDF) Bioremediation Consortium has conducted cometabolic bioventing demonstrations at Dover and Hill Air Force Bases. At Dover, a field demonstration of cometabolic bioventing was conducted at Building 719—a site contaminated with fuel and solvents during engine inspection and maintenance operations. The targeted contaminants of the demonstration and their concentrations were TCE, as high as 250 mg/kg; 1,1,1-TCA, 10–1,000 mg/kg; and cis-1,2-DCE, 1–20 mg/kg. Laboratory tests were used to select propane as the cometabolic substrate and to predict that a substrate acclimation period would be needed. The test plot was acclimated to propane addition through pulsed propane/air injections for three months, then the test plot was operated for 14 months with continuous propane injection. Concentrations of TCE, TCA, and DCE were reduced to less than 0.25, 0.5, and 0.25 mg/kg, respectively. Soil chloride accumulation analysis confirmed biodegradation as the mechanism of removal (EPA, 1998a).

As with aerobic bioventing, difficulty in distributing gases in the subsurface may make the application of cometabolic bioventing more complicated. In some cases, such as high moisture content or low-permeability soils, the design of the cometabolic system may compensate for poor permeability. In the case of shallow contamination, designing a cometabolic bioventing system that minimizes environmental release and achieves sufficient aeration and organic substrate distribution may be difficult (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003).

Another limitation to cometabolic bioventing is the lack of experience with the technology. Since cometabolic bioventing has been demonstrated at a limited number of sites, the technology is not as well understood as aerobic bioventing. Researchers are still studying which contaminants are amenable to this type of biodegradation and what removal rates can be expected. Establishing that biological processes are the primary mechanism for contaminant removal is also more difficult. Finally, regulatory and public acceptance is not as strong for cometabolic bioventing as for aerobic bioventing. However, treatability testing of samples from the contaminated site and pilot-scale testing may alleviate many of these limitations and concerns (EPA, 1998a). As more sites are remediated using cometabolic bioventing, these limitations may ease.

3.1.2.1.3 Anaerobic Bioventing

While aerobic and cometabolic bioventing are useful for degrading many hydrocarbons and lightly chlorinated compounds, some chlorinated species are not effectively treated aerobically. Microbes may degrade these contaminants directly via anaerobic reductive dechlorination or through anaerobic cometabolic pathways. Anaerobic reductive dechlorination is a biological mechanism typically marked by sequential removal of chlorine from a molecule. Microbes possessing this pathway do not gain energy from this process. Anaerobic cometabolism is similar to aerobic cometabolism in that microbes fortuitously degrade contaminants while reducing other compounds (cometabolites). Anaerobic bioventing may use both biological mechanisms to destroy the contaminants of concern.

Anaerobic bioventing uses the same type of gas delivery system as the other bioventing technologies, but injects nitrogen and an electron donor, instead of air, to establish reductive anaerobic conditions. The nitrogen displaces the soil oxygen, and small amounts of an electron donor gas (such as hydrogen and carbon dioxide) produce reducing conditions in the subsurface, thereby facilitating microbial dechlorination. Volatile and semi-volatile compounds may be produced during anaerobic bioventing. Some of these compounds may be slow to degrade under anaerobic conditions. These compounds may be treated in two ways. Volatile compounds may diffuse into the soils surrounding the treatment zone, where aerobic degradation may occur. SVOCs and VOCs remaining in the treatment zone may be treated by following anaerobic bioventing with aerobic bioventing. Since aerobic and anaerobic bioventing share similar gas delivery systems, the switch can be made by simply changing the injected gas.

Anaerobic bioventing is an emerging technology that has been demonstrated in several laboratory and field studies. This process may be useful in treating highly chlorinated compounds such as tetrachloroethene (PCE), TCE, RDX, pentachlorophenol, and pesticides such as lindane and dichlorodiphenyltrichloroethane (DDT). Due to the limited experience with this technique, laboratory, pilot, and field demonstrations are recommended to confidently apply this technology to remediate a site.

As with the other bioventing technologies, the ability to deliver gases to the subsurface is important. Soils with high moisture content or low gas permeability may require careful system design to deliver appropriate levels of nitrogen and the electron donor. Sites with shallow contamination or nearby buildings are also a challenge since this technology is operated by injecting gases. In addition, anaerobic bioventing can take a few years to clean up a site depending on the contaminant concentrations and site-specific removal rates. If a quicker cleanup is needed, other technologies may be more appropriate.

3.1.2.2 Surficial Soil Remediation

If contamination is shallow, soil may be treated in place using techniques similar to land treatment or composting. Variations of these technologies involve tilling shallow soils and adding amendments to improve aeration and bioremediation. This process is similar to the land farming and composting discussed later in the *Ex Situ* Bioremediation section of this Engineering Issue paper, except that the soils are not excavated.

Since these treatments do not include an impermeable sublayer, contaminant migration may be a concern depending on the contaminants of concern and treatment amendments. A more prudent approach would be to excavate soils and treat them in lined beds.

This technology will generally require special permission from the applicable regulatory agency. Frequently, some type of monitoring for contaminant migration is required.

3.1.2.3 Ground Water and Saturated Soil Remediation

In situ bioremediation techniques applicable to ground water and saturated soil include dechlorination using anaerobic reducing conditions, enhanced aerobic treatment, biological reactive barriers that create active remediation zones, and bioslurping/biosparging techniques that promote aerobic degradation.

3.1.2.3.1 Anaerobic Reductive Dechlorination

Anaerobic reductive dechlorination has been used at many sites where the ground water has been contaminated with chlorinated solvents, such as TCE or PCE. In this treatment, organic substrates are delivered to the subsurface where they are fermented. The fermentation creates an anaerobic environment in the area to be remediated and generates hydrogen as a fermentation byproduct. The hydrogen is used by a second microbial population to sequentially remove chlorine atoms from chlorinated solvents (AFCEE, 2004). If PCE were degraded via reductive dechlorination, the following sequential dechlorination would be observed: PCE would be converted to TCE, then to DCE, vinyl chloride (VC), and/or dichloroethane (EPA, 1998a).

Anaerobic dechlorination may also occur via cometabolism where the dechlorination is incidental to the metabolic activities of the organisms. In this case, contaminants are degraded by microbial enzymes that are metabolizing other organic substrates. Cometabolic dechlorination does not appear to produce energy for the organism. At pilot- or full-scale treatment, cometabolic and direct dechlorination may be indistinguishable, and both processes may contribute to contaminant removal. The microbial processes may be distinguished in the more controlled environment of a bench-scale system (EPA, 1998a).

Anaerobic reductive dechlorination is primarily used to treat halogenated organic contaminants, such as chlorinated solvents. As well as the variables discussed initially, the treatment rate and system design are dependent on several factors including site hydrology and geology, type and concentration of organic substrates, and site history. As with cometabolic bioventing, the selection of organic substrate and the concentration used are controllable and can be important to the removal rate. Treatability or bench-scale testing can be useful in selecting the best organic substrate and concentration for a site. In addition, small-scale testing can demonstrate that full dechlorination is possible at a site. In some cases, dechlorination may stall at DCE despite the presence of sufficient electron donors. If a site does not demonstrate full dechlorination (either as part of site assessment or in microcosm testing), a combined treatment strategy, such as anaerobic treatment followed by aerobic treatment, may be successful. Alternatively, bioaugmentation may improve the dechlorination rate (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003). Research methods used to establish that anaerobic dechlorination is occurring at a site are similar to those discussed in previous sections of this Engineering Issue paper.

Regulatory considerations for this technology involve the Safe Drinking Water Act and RCRA hazardous waste regulations, as well as state and local regulations. At the time that this Engineering Issue paper was written, judgments about the applicability of this technology were made on a case-by-case basis. The regulations can impact the design and operation of the treatment system as well as the overall applicability. *Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications* (EPA, 2000) provides more detailed information about regulatory concerns and applicability (EPA, 1998a).

Additional information on anaerobic reductive dechlorination is available from the following source: *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* (AFCEE, 2004).

3.1.2.3.2 Aerobic Treatment

Similar to bioventing, enhanced *in situ* aerobic ground water bioremediation processes are used in situations where aerobically degradable contaminants, such as fuels, are present in anaerobic portions of an aquifer. In these situations, air or other oxygen sources are injected into the aquifer near the contamination (see Figure 3-2). As the oxygenated water migrates through the zone of contamination, the indigenous bacteria are able to degrade the contaminants (EPA, 1998a; EPA, 2000).

Aerobic treatment may also be used to directly or cometabolically degrade lightly chlorinated species, such as DCE or VC. In the direct aerobic pathway, air is injected into the aquifer. The microbes appear to generate energy by oxidizing the hydrocarbon backbone of these contaminants, resulting in the release of chloride (EPA, 2000).

This process has been used to complete contaminant removal following anaerobic treatment at several sites (EPA, 1998a; EPA, 2000).

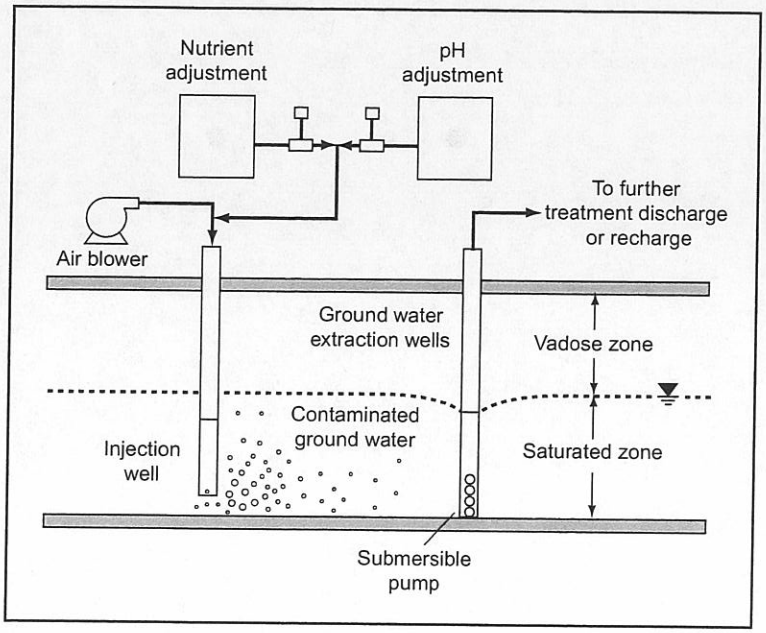

Figure 3-2. Aerobic treatment. (Adapted from FRTR, 2003)

Cometabolic aerobic treatment is founded on the same biological principles as cometabolic bioventing and involves the addition of oxygen and organic substrates, such as methane, to the aquifer. As with other cometabolic processes, these organic substrates are metabolized by enzymes that incidentally degrade the contaminant. In this treatment, sufficient oxygen must be present to fuel the oxidation of both the substrate and contaminant (AFCEE, 1996; EPA, 1998a).

3.1.2.3.3 Amendment Delivery

In situ ground water treatment, either aerobic or anaerobic, may be configured as direct injection of air or aqueous streams or as ground water recirculation. In direct injection, amendments, such as organic substrates, oxygen sources, or nutrients, are directly injected into the aquifer. For example, oxygen may be sparged into the aquifer as a gas. Lactate or hydrogen peroxide may be injected as a liquid stream; when using hydrogen peroxide, caution should be used as it may act as a disinfectant. In some cases, both liquids and gases are added. The ground water recirculation configuration involves extracting ground water, amending it as needed, and then re-injecting it back into the aquifer. Recirculation may also be conducted below the ground surface by extracting ground water at one elevation, amending it in the ground, and re-injecting it into another elevation (EPA, 1998a; EPA, 2000).

In addition to the variables discussed initially, the treatment rates and system design are the result of several factors including site hydrology and geology, amendment to be added, solubility of air or oxygen sources, and site history. The low solubility of air in water often limits reaction rates and may make this process impractical if cleanup time is short (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003).

Careful attention also should be given to co-contaminants, especially metals. When an aquifer environment is converted from an aerobic to an anaerobic environment, a variety of chemical species may become soluble. Therefore, it is important to check for changes in co-contaminants such as arsenic, which may be solubilized during the treatment process (AFCEE, 1996; EPA, 1998a; EPA, 2000; FRTR, 2003).

3.1.2.3.4 Biological Reactive Barriers

Biological reactive barriers consist of an active bioremediation zone created in the contamination zone. These barriers may be constructed to exploit aerobic or anaerobic processes depending on the contaminant of concern and site needs. A trench is excavated and filled with sand pre-mixed with nutrient-, oxidant-, or reductant-rich materials to form a bioremediation zone (see Figure 3-3). Alternatively, a bioremediation curtain can be formed by injection of amendments or recirculation of amended ground water at the toe of the plumes (EPA, 2000). Contaminants biodegrade as they pass through the permeable reactive barrier (PRB).

Trenches are dug with a backhoe or similar device and are filled with permeable materials, such as sand or bark mulch, that are mixed together prior to placement. Nutrients, degradable carbonaceous substrates (e.g., manure, compost, and wheat straw), and other additives are introduced into the permeable layer. As ground water flows through the treatment zone, indigenous microbes are stimulated to improve natural biodegradation (NFESC, 2000). Biological PRBs have been studied by the PRB RTDF, and further information, including case studies, may be found at http://www.rtdf.org/public/permbarr/pbar_qa.htm and http://costperformance.org/search.cfm.

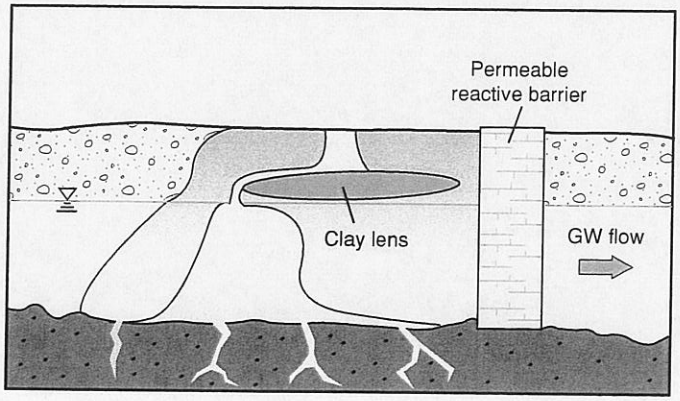

Figure 3-3. Permeable reactive barrier. (Adapted from EPA, 2000)

3.1.2.3.5 Biosparging and Bioslurping

Biosparging (similar to air sparging) involves the injection of a gas (usually air or oxygen) and occasionally gas-phase nutrients, under pressure, into the saturated zone to promote aerobic biodegradation (GWRTAC, 1996). In air sparging, volatile contaminants also can be removed from the saturated zone by desorption and volatilization into the air stream. Emphasis on the biological degradation rate over physical removal, as well as lower rates of air injection, are what distinguishes this technology from air sparging.

Typically, biosparging is achieved by injecting air into a contaminated subsurface formation through a specially designed series of injection wells. The air creates an inverted cone of partially aerated soils surrounding the injection point. The air displaces pore water, volatilizes contaminants, and exits the saturated zone into the unsaturated zone. While in contact with ground water, oxygen dissolution from the air into the ground water is facilitated and supports aerobic biodegradation.

A number of contaminants have been successfully addressed with biosparging technology, including gasoline components such as benzene, toluene, ethylbenzene, and xylenes (BTEX) and SVOCs. Biosparging is most often recommended at sites impacted with mid-weight petroleum hydrocarbon contaminants, such as diesel and jet fuels. Lighter contaminants, such as gasoline, tend to be easily mobilized into the unsaturated zone and physically removed. Heavier contaminants, such as oils, require longer remedial intervals because of reduced microbial bioavailability with increasing carbon chain length (EPA, 2004b).

Care must be taken to determine whether contaminant concentrations in soil gas and released vapors resulting from biosparging require treatment. For this reason, biosparging may be implemented along with SVE or bioventing as a remedy for increased contaminant concentrations in the unsaturated zone. The SVE wells are designed to capture the introduced air and contaminant vapors (EPA, 2004b). Figure 3-4 depicts a typical biosparging system with optional SVE system. Alternatively, a lower-flow bioventing system may be added to facilitate bioremediation of volatilized contaminants in the vadose zone.

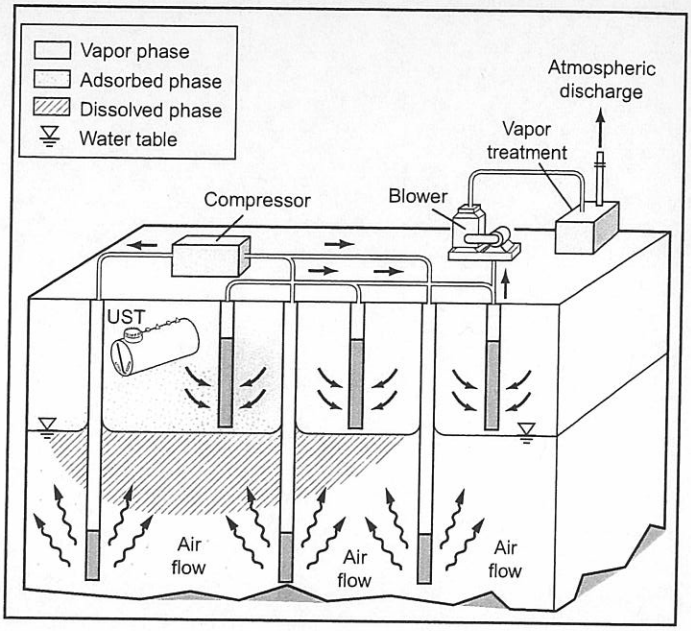

Figure 3-4. Biosparging system (used with soil vapor extraction). (Adapted from NMED, 2004)

One specialized form of biosparging involves the injection of organic gases into the saturated zone to induce cometabolic biodegradation of chlorinated aliphatic hydrocarbons, and this is analogous to cometabolic bioventing discussed in this Engineering Issue paper. The injection of gases below the water table distinguishes biosparging from bioventing.

In contrast to cometabolic bioventing, the solubility of organic gases in water limits delivery of the primary substrate during cometabolic biosparging applications. This solubility limitation affects the economics of cometabolic biosparging applications since the interaction between bacterial cometabolite consumption and cometabolite water solubility directly determines the number of methane biosparging injection wells required at a given site. Safety precautions similar to those required for cometabolic bioventing apply to cometabolic biosparging (Sutherson, 2002; AFCEE, 1998).

Bioslurping (also known as multi-phase extraction) is effective in removing free product that is floating on the water table (Battelle, 1997). Bioslurping combines the two remedial approaches of bioventing and vacuum-enhanced free-product recovery. Bioventing stimulates aerobic bioremediation of contaminated soils *in situ*, while vacuum-enhanced free-product recovery extracts light, nonaqueous-phase liquids (LNAPLs) from the capillary fringe and the water table (AFCEE, 2005). Bioslurping is limited to 25 feet below ground surface as contaminants cannot be lifted more than 25 feet by this method.

A bioslurping tube with adjustable height is lowered into a ground water well and installed within a screened portion at the water table (see Figure 3-5). A vacuum is applied to the bioslurping tube and free product is "slurped" up the tube into a trap or oil water separator for further treatment. Removal of the LNAPL results in a decline in the LNAPL elevation, which in turn promotes LNAPL flow from outlying areas toward the bioslurping well. As the fluid level in the bioslurping well declines in response to vacuum extraction of LNAPL, the bioslurping tube also begins to extract vapors from the unsaturated zone. This vapor extraction promotes soil gas movement, which in turn increases aeration and enhances aerobic biodegradation (Miller, 1996).

Recent improvements in bioslurping technology and application assessments for this technology are contained in reports by the U.S. Navy (NFESC, 2003) and the U.S. Army Corps of Engineers (USACE, 1999). It is widely accepted in the industry that source removal, such as that offered by proper application of the bioslurping technology, should be part of most remedial strategies at sites involving separate product phases.

3.2 *Ex Situ Bioremediation*

Ex situ bioremediation technologies can most easily be classified by the physical state of the medium to which they are typically applied. The following discussion is organized accordingly, with descriptions of bioremediation processes for various solids, solid–liquid mixtures, and liquids.

Also common to the *ex situ* remediation technologies are the processes for removing contaminated materials for treatment. Contaminated media are excavated or extracted (e.g., ground water removal by pumping) and moved to the process location, which may be within or adjacent to the contamination zone. Special handling required for excavation and preparation of sediments is described in detail in *Selecting Remediation Techniques for Contaminated Sediment* (EPA, 1993b), as well as *Physical Separation (Soil Washing) for Volume Reduction of Contaminated Soils and Sediments: Processes and Equipment* (Olin et al., 1999).

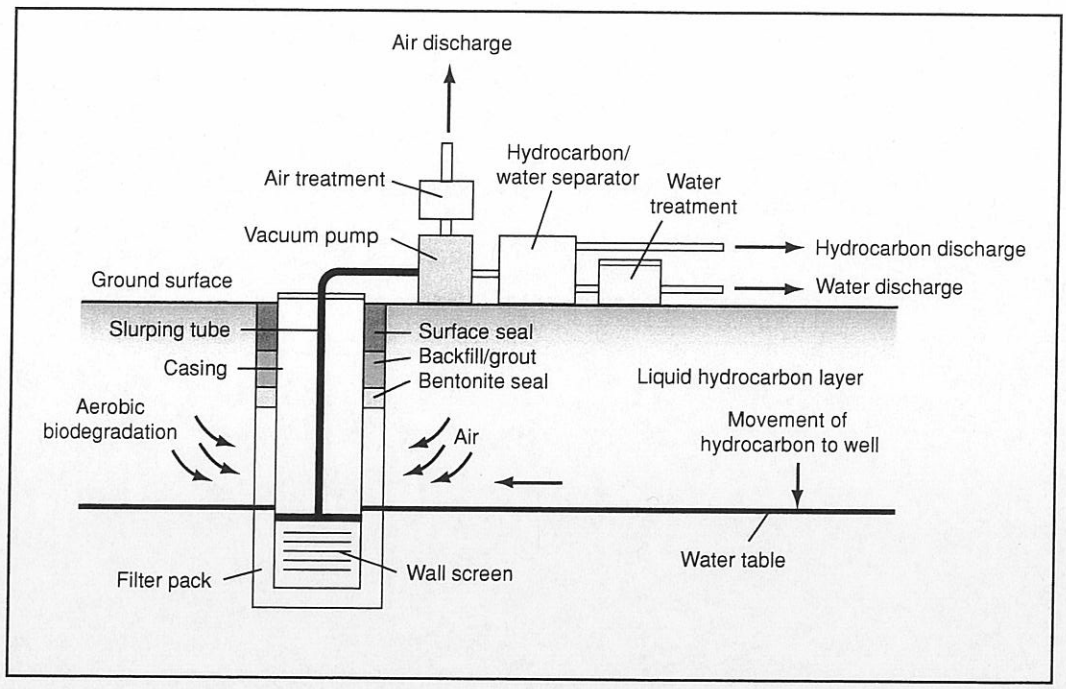

Figure 3-5. Bioslurping technology. (Adapted from USAEC, 2004)

3.2.1 Solids

The most common types of solids bioremediation are (1) land farming or land treatment, (2) composting, and (3) biopiles, cells, or mounds. In practice, these types are not rigidly divided, but the subdivision is useful for this discussion. Table 3-1 presents a comparison of the characteristics of various solid-phase bioremediation technologies.

Table 3-1. Comparison of the Characteristics of *Ex Situ* Bioremediation Technologies for Solids

Characteristic	Land Treatment	Composting			Biopiles
		Static Pile	In-Vessel	Windrow	
Liner Utilized	Yes	Yes	No	Yes	Yes
Containerized	Bermed	Pad	Yes	Pad	Pad
Bulking Agent	Sometimes	Yes	Yes	Yes	Sometimes
Mechanical	Mobile Equipment	No	In-Vessel	Mobile Equipment	Mobile Equipment
Aeration[2]	Mechanical Mixing	Pressure	Vacuum	Mechanical	Pressure/Vacuum
Nutrient[3] Added	Sometimes	Yes	Yes	Yes	Yes
Temperature	Ambient	54°–65°C	54°	65°C	Ambient/Mesophilic

[1] Mechanical mixing may be necessary to provide thorough distribution of nutrients and other additives, promote aeration, and enhance biodegradation. Mechanical mixing may use mobile equipment (e.g., bulldozers) or may occur within a reactor vessel.
[2] Aeration involves the introduction of oxygen into *ex situ* material to promote aerobic degradation. Aeration mechanisms may include mechanical mixing, pressure, and vacuum, as well as natural draft. "Typical" aeration mechanisms are shown.
[3] Nutrients may not be required or may be supplied by bulking agents alone, or additional nutrients may be supplied.

3.2.1.1 Land Treatment

Land treatment, also called land farming, is useful in treating aerobically degradable contaminants. This process is suitable for non-volatile contaminants at sites where large areas for treatment cells are available. Land treatment of site-contaminated soil usually entails the tilling of an 8- to 12-inch layer of the soil to promote aerobic biodegradation of organic contaminants. The soils are periodically tilled to aerate the soil, and moisture is added when needed. In some cases, amendments may be added to improve the tilth of the soil, supply nutrients, moderate pH, or facilitate bioremediation. Typically, full-scale land treatment would be conducted in a prepared-bed land treatment unit (see Figure 3-6)—an open, shallow reactor with an impermeable lining on the bottom and sides to contain leachate, control runoff, and minimize erosion and with a leachate collection system under the soil layer (EPA, 1993). In some cases, hazardous wastes (such as highly contaminated soils) or process wastes (such as distillate residues) may be treated in land treatment units. In these cases, the waste may be applied to a base soil layer.

The performance of land treatment varies with the contaminants to be treated. For easily biodegradable contaminants, such as fuels, land treatment is inexpensive and effective. Contaminants that are difficult to degrade, such as PAHs, pesticides, or chlorinated organic compounds, are topics of research and would require site-specific treatability testing to verify that land treatment can meet desired endpoints.

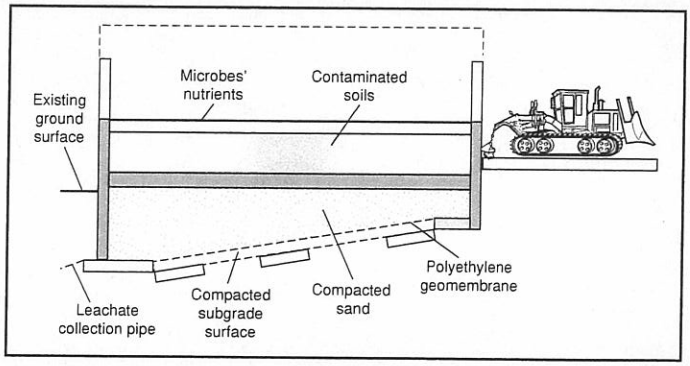

Figure 3-6. Land treatment. (Adapted from FRTR, 2003)

3.2.1.2 Composting

Composting is a controlled biological process that treats organic contaminants using microorganisms under thermophilic conditions (40°–50°C). For some practitioners, the creation of thermophilic conditions is the primary distinction between composting and biopiles (which operate at less than 40°C), although others use composting as a term that encompasses both temperature ranges (NFESC, 2005).

In composting, soils are excavated and mixed with bulking agents and organic amendments, such as wood chips and vegetative wastes, to enhance the porosity of the mixture to be decomposed. Degradation of the bulking agent heats up the compost, creating thermophilic conditions. Oxygen content, moisture levels, and temperatures are monitored and manipulated to optimize degradation. Oxygen content usually is maintained by frequent mixing, such as daily or weekly turning of windrows. Sur-

face irrigation often is used to maintain moisture content. Temperatures are controlled, to a degree, by mixing, irrigation, and air flow, but are also dependent on the degradability of the bulk material and ambient conditions (FRTR, 2003).

There are three designs commonly applied for composting:

1. *Aerated static piles*—Compost is formed into piles and aerated with blowers or vacuum pumps.
2. *Mechanically agitated in-vessel composting*—Compost is placed in a reactor vessel, in which it is mixed and aerated.
3. *Windrow composting*—Compost is placed in long, low, narrow piles (i.e., windrows) and periodically mixed with mobile equipment.

Windrow composting is the least expensive method, but has the potential to emit larger quantities of VOCs (FRTR, 2003). In-vessel composting is generally the most expensive type, but provides for the best control of VOCs. Aerated static piles, especially when a vacuum is applied, offer some control of VOCs and are typically in an intermediate cost range, but will require offgas treatment (FRTR, 2003).

Berms may also be needed to control runoff during composting operations. Runoff may be managed by retention ponds, provision of a roof, or evaporation.

Composting has been successfully applied to soils and biosolids contaminated with petroleum hydrocarbons (e.g., fuels, oil, grease), solvents, chlorophenols, pesticides, herbicides, PAHs, and nitro-aromatic explosives (EPA, 1998b; EPA, 1997; EPA, 2004b). For TNT, complete mineralization has been difficult to demonstrate via composting. TNT may bind to soil, resulting in low microbial bioavailability and apparent disappearance (Rodgers and Bunce, 2001). Composting is not likely to be successful for highly chlorinated substances, such as PCBs, or for substances that are difficult to degrade biologically (EPA, 1998b).

3.2.1.3 Biopiles

Biopiles involve the mixing of excavated soils with soil amendments, with the mixture placed in a treatment area that typically includes an impermeable liner, a leachate collection system, and an aeration system. Biopiles are typically 2–3 meters high, and contaminated soil is often placed on top of treated soil (see Figure 3-7). Moisture, nutrients, heat, pH, and oxygen are controlled to enhance biodegradation. This technology is most often applied to readily degradable species, such as petroleum contaminants. Surface drainage and moisture from the leachate collection system are accumulated, and they may be treated and then recycled to the contaminated soil. Nutrients (e.g., nitrogen and phosphorus) are often added to the recycled water. Alkaline or acidic substances may also be added to the recycled water to modify or stabilize pH to optimize the growth of select microbes capable of degrading the contaminants of concern (FRTR, 2003).

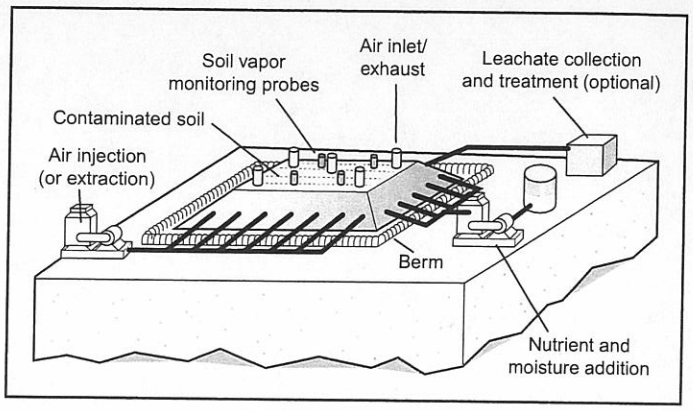

Figure 3-7. Typical biopile system. (Adapted from EPA, 2004b)

An air distribution system is buried in the soil as the biopile is constructed. Oxygen exchange can be achieved utilizing vacuum, forced air, or even natural draft air flow. Low air flow rates are desirable to minimize contaminant volatilization. If volatile constituents are present in significant concentrations, the biopile may require a cover and treatment of the offgas.

Biopile treatment lasts from a few weeks to a few months, depending on the contaminants present and the design and operational parameters selected for the biopile (FRTR, 2003). Biopiles are typically mesophilic (10°–45°C).

Additional information on *ex situ* biological soil treatment is available from the following sources:

- U.S. Navy *Biocell Application Guidance* (NFESC, 1998)
- U.S. Navy *Biopile Design and Construction Manual* (Battelle, 1996a)
- U.S. Navy *Biopile Operations and Maintenance Manual* (Battelle, 1996b)
- U.S. Army *Environmental Center Multiple Biotechnology Demonstration of Explosives-Contaminated Soils* (USAEC, 2005)

- Chapters 13 and 14 in *Biodegradation of Nitroaromatic Compounds and Explosives* (Spain et al., 2000)
- *On-site Bioremediation of Oil and Grease Contaminated Soils* (Vance, 1991).

3.2.2 Solid–Liquid Mixtures

Solid-liquid mixtures consist of materials such as slurries and sludges. One technology for treating such mixtures is discussed below.

3.2.2.1 Slurry Bioreactors

Slurry bioreactors are utilized for soil, sediments, sludge, and other solid or semi-solid wastes. Slurry bioreactors are costly and, thus, are likely to be used for more difficult treatment efforts.

Typically, wastes are screened to remove debris and other large objects, then mixed with water in a tank or other vessel until solids are suspended in the liquid phase. If necessary, further particle size reduction can be accomplished before the addition of water (by pulverizing and/or screening the wastes) or after the addition of water (through use of a sheering mixer). Suspension and mixing of the solids may increase mass transfer rates and may increase contact between contaminants and microbes capable of degrading those contaminants (EPA, 1990).

Mixing occurs in tanks or lined lagoons. Mechanical mixing is generally conducted in tanks. Typical slurries are 10–30% solids by weight (FRTR, 2003). Aeration, with submerged aerators or spargers, is frequently used in lagoons and may be combined with mechanical mixing to achieve the desired results. Nutrients and other additives, such as neutralizing agents, surfactants, dispersants, and co-metabolites (e.g., phenol, pyrene) may be supplied to improve handling characteristics and microbial degradation rates. Indigenous microbes may be used or microorganisms may be added initially to seed the bioreactor or may be added continuously to maintain proper biomass levels. Residence time in the bioreactor varies with the matrix as well as the type and concentration of contaminant (EPA, 1990).

Once contaminant concentrations reach desired levels on a dry-weight basis, the slurry is dewatered. Typically, a clarifier is utilized to dewater the slurry by gravity. Other dewatering equipment may be used depending on slurry characteristics and cost considerations (Olin et al., 1999). Water, air emissions from all process steps, and oversize materials may require additional treatment. More information on this technology is available from the Naval Facilities Engineering Service Center (NFESC) at http://enviro.nfesc.navy.mil/erb/erb_a/restoration/technologies/tech_transfer/ttweb.asp?id=3.

3.2.3 Liquids

Liquids, such as surface water, ground water, mine drainage, and effluent from other treatment operations, can undergo *ex situ* bioremediation in constructed wetlands. Note that surface water and ground water have important differences, such as concentrations of contaminants and degradable organic material, than may be found in waste streams from other treatment operations.

3.2.3.1 Constructed Wetlands

Constructed wetlands provide for biological assimilation, breakdown, and transformation of contaminants; chemical breakdown and transformation of contaminants; and physical sedimentation and filtration (USDA and EPA 1994a), as shown in Figure 3-8. Biological processes associated with wetlands include bioremediation (microbially-based remediation) and phytoremediation (plant-based remediation). Microbes attached to the surfaces of plants, plant litter, and the wetland substrate degrade and/or sorb the organic substances present in the water undergoing treatment (USDA and EPA, 1994a). Phytoremediation uses plants to remove, transfer, stabilize, or destroy contaminants through biological, chemical, and physical processes that are influenced by plants and their roots (i.e., rhizosphere) that include degradation, extraction through accumulation in plant roots/shoots/leaves, metabolism of contaminants, and immobilization of contaminants at the interface of roots and soil (EPA, 2004a).

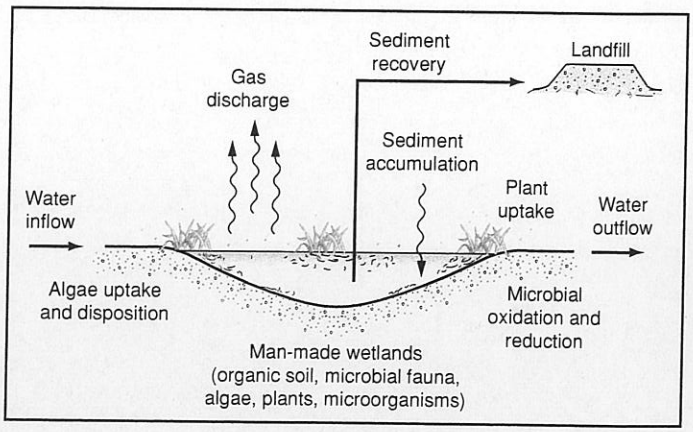

Figure 3-8. Constructed wetland. (Adapted from FRTR, 2003)

Wetlands inherently have a higher rate of biological productivity/activity than many other natural ecosystems and are thus capable of efficiently and economically transforming many common contaminants to harmless byproducts (Kadlec and Knight, 1996). Constructed wetlands have been applied successfully to remove contaminants such as metals, petroleum hydrocarbons, and glycols; to decrease metal concentrations via chemical or microbial precipitation; and to neutralize acidity (ESTCP, 2004a; USDA and EPA, 1994b). Recent research also has demonstrated applicability to explosive-contaminated water (Bader, 1999). However, wetlands are sensitive to high ammonia levels, herbicides, and contaminants that are toxic to the plants or microbes (USDA and EPA, 1994a).

Constructed wetlands are well suited for the treatment of contaminated ground water emerging from surface and mine seeps, pump-and-treat waste streams with low concentrations of easily biodegradable contaminants, and contaminated surface waters (EPA, 2001c). Constructed wetlands may also be used to pretreat contaminated water prior to conventional treatment or to further treat a waste stream prior to disposition or discharge (USDA and EPA, 1994b). However, applicability to highly acidic waste streams may not be cost-effective (USDA and EPA, 1994b).

Discharges must meet applicable effluent limitations and related regulatory requirements. Discharges that do not meet these requirements may be required to undergo further treatment or may be found suitable for recycling into the wetland as a supplemental water source (USDA and EPA, 1994a).

There are various types of constructed wetlands, depending on the type of flow (surface or subsurface), contaminant of concern, or type of substrate, which can include limestone, organic material such as compost, or gravel (USDA and EPA, 1994a; USDA and EPA, 1994b; Bader, 1999). The chemical and microbial processes may proceed either in an anaerobic or aerobic environment.

Since constructed wetlands function both as macroscopic and microscopic ecosystems to promote contaminant treatment, the biological characteristics of the system must be taken into account during the design phase. The chemistry of the waste stream and how the passive chemical, physical, and biological processes affect this or are, in turn, affected by the waste stream are important design factors (USDA and EPA, 1994b). The chemical characteristics of the waste stream can affect sizing of the system for adequate retention time and whether the waste stream may require pretreatment to (1) address concentration, ammonia, nutrient, and organic loads that may damage vegetation, or (2) remove solids or materials, such as grease, that may clog the wetland (USDA and EPA, 1994a). In addition, pH adjustment may be necessary, either prior to waste stream treatment or through use of limestone substrate (USDA and EPA, 1994b).

Climatic and seasonal circumstances as well as waste stream characteristics are important considerations when selecting the types of plants to use in a constructed wetland. Salinity, either in the waste stream or as a result of treatment, can harm or destroy the wetland vegetation if the plants are not salt tolerant. In addition, cold weather can reduce microbial activity, and hail or other weather events can damage the plants (USDA and EPA, 1994a).

The low cost, passivity (i.e., lack of dependence on power or mechanical components), and efficacy for treating many common contaminants are key advantages of constructed wetland treatment systems. Constructed wetlands are often visually attractive, but can require more space than other remedial systems. The wetlands should be sized with an understanding that both plant-based and bacterial-based remediation will decline during colder seasons. A key design element is sizing to achieve adequate retention time to enable the biological, chemical, and physical processes to be effective (USDA and EPA, 1994a). Seasonal, climatological, and waste stream factors that control the water balance in the wetland also must be considered during design to achieve project goals (FRTR, 2003). For example, photosynthesis of TNT colors the treated water red, which negatively impacts plant growth (Bader, 1999), and pH affects the kinetics of the abiotic and biotic processes, including solubility of metal oxides or hydroxides, oxidation, and hydrolysis (USDA and EPA 1994b). In addition, animals such as tadpoles or deer may defoliate plant material, thereby affecting treatment (Bader, 1999).

Constructed wetlands require a continuous supply of water. While tolerant of fluctuating flows, constructed wetlands cannot withstand complete drying. A slow water flow must be maintained to prevent the development of stagnant water that can lead to performance and vector difficulties. Recycling wetland water can supplement inflow, but this can increase salinity over time, which can affect design and cost (USDA and EPA, 1994a).

More information on constructed treatment wetlands is available in the Interstate Technical and Regulatory Guidance Document *for Constructed Wetlands* (ITRC 2003).

4.0 TECHNOLOGY SELECTION FACTORS

Table 4-1 summarizes the general applicability of each technology type for the contaminant classifications discussed in this Engineering Issue. The table presents information for contaminant treatment in soil, sediment, sludge, ground water, surface water, and leachate. The analysis of technology applicability is based on published literature and expert judgment. Note that the technologies may be applicable to some contaminants within a contaminant group but not to others. Site- and contaminant-specific treatability studies may be required to determine the actual efficacy of any one technology on the site-specific soils, contaminants, and conditions.

Applicability of biologically based remedies is highly influenced by the type of microbiological community that is best suited for the biodegradation of the particular contaminant or mix of contaminants. Of primary importance in gaining an initial understanding of the applicability of a biological remedy to contaminants of concern is the baseline oxidation/reduction potential of the site to be remediated. The presence or absence of oxygen is a significant determining factor that defines the microbiological community characteristics. Some contaminants are best treated under anaerobic conditions, and others can only be treated aerobically. Some contaminants can be treated both anaerobically and aerobically, but there are technical implications to be considered and contaminant half-lives may vary between the two processes. Therefore, the baseline oxidation/reduction potential is of primary importance to the practitioner who is evaluating a contaminated site for a biological remedy (Rottero et al., 2004).

Table 4-1. Demonstrated Effectiveness of Biological Treatment Technologies for Soil, Sediment, Bedrock, and Sludge

Contaminant Type	In Situ Treatment Technologies										Ex Situ Treatment Technologies[1]				
	Intrinsic	Enhanced													
	Soil / Ground Water	Vadose Zone			Surficial Soil		Ground Water / Saturated Soil				Solids			Solid–Liquid Mixtures	Liquids
	Monitored Natural Attenuation	Aerobic Bioventing	Cometabolic Bioventing	Anaerobic Bioventing	Land Treatment	Composting	Anaerobic Reductive Dechlorination	Aerobic Treatment	Biological Reactive Barriers	Biosparging/ Bioslurping	Land Treatment	Composting	Biopiles	Slurry Bioreactors	Constructed Wetlands
Non-halogenated VOCs	◆	◆	■	■	◆[2]	◆[2]	■	◆	◆	◆	◆[2]	◆[2]	◆[2]	◆[2]	◆[2]
Halogenated VOCs	◆	■	◆	▲	■[2]	■	◆	■	◆	◆	■[2]	■[2]	■[2]	■[2]	■[2]
Non-halogenated SVOCs	▲	◆	■	■	◆	◆	■	◆	▲	▲	◆	◆	◆	◆	◆
Halogenated SVOCs	▲	■	▲	▲	■	■	▲	■	◆	■	■	■	■	■	■
Fuels	◆	◆	■	■	◆[2]	◆[2]	■	◆	◆	◆	◆[2]	◆[2]	◆[2]	◆[2]	◆[2]
Inorganics	■	●	●	■	■	■	■	■	◆	■	■	■	■	■	◆
Radionuclides	■	■	■	■	■	■	■	■	◆	■	■	■	■	■	■
Explosives	■	▲	■	▲	◆	◆	■	▲	◆	■	◆	◆	▲	◆	▲

[1] Not generally applicable to rocks and bedrock.
[2] Volatilization must be controlled.

◆ **Demonstrated Effectiveness:** Successfully treated at pilot or full scale and verified by an independent agency.

▲ **Potential Effectiveness:** Successfully treated at laboratory or bench scale, or similar contaminant types have been successfully demonstrated at pilot or full scale.

■ **No Expected Effectiveness:** No successful treatments documented at any scale, and expert opinion notes that the contaminant in question is not likely to be effectively treated by the technology.

● **Potential Adverse Effects:** Adverse effects are documented at any scale, or expert opinion notes that the treatment technology may result in adverse effects to the environment.

Adapted from information in EPA (1998a, 2000, 2004b, 2004c), FRTR (2003, 2004), ESTCP (2001, 2004a, 2004b), ITRC (2004), and AFCEE (1996).

Site characterization and long-term monitoring are necessary to support system design and sizing as well as to verify continued performance. There are also regulatory requirements to be addressed regarding system design, implementation, operation, and performance, including the disposition of liquid effluents and other wastes resulting from the treatment process.

5.0 SUMMARY

In situ and *ex situ* biodegradation technologies are increasingly selected to remediate contaminated sites, either alone or in combination with other source control measures. Bioremediation technologies have proven effective in remediating fuels and VOCs and are often able to address diverse organic contaminants including SVOCs, PAHs, CAHs, pesticides and herbicides, and nitro-aromatic compounds (such as explosives), potentially at lower cost than other remediation options. Some bioremediation techniques are also able to address heavy metal contamination. Bioremediation continues to be an active area of research, development, and demonstration for its applications to diverse contaminated environments.

A unique feature of bioremediation is the diversity of its application to solids, liquids, and liquid–solid mixtures, involving both *in situ* and *ex situ* environments. Amendments may be necessary to support or enhance the biodegradation processes to improve the timeframe involved to achieve cleanup goals.

Site characterization and long-term monitoring are necessary to support system design and sizing as well as to verify continued performance. There are also regulatory requirements to be addressed regarding system design, implementation, operation, and performance, including the disposition of liquid effluents and other wastes resulting from the treatment process.

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