

Technology  
Overview Report

TO-98-01



---

# *In Situ* Bioremediation

---

Prepared By:

**Liesbet van Cauwenberghe**

and

**Diane S Roote, P.G.**

Ground-Water Remediation  
Technologies Analysis Center

October 1998

Prepared For:



*Ground-Water Remediation  
Technologies Analysis Center*

320 William Pitt Way • Pittsburgh, PA 15238 • (412) 826-5320 Ext 215 • (800) 373-1973  
Homepage: <http://www.gwrtac.org> E-mail: [gwrtac@gwrtac.org](mailto:gwrtac@gwrtac.org)

---

## FOREWORD

### **About GWRTAC**

The Ground-Water Remediation Technologies Analysis Center (GWRTAC) is a national environmental technology transfer center that provides information on the use of innovative technologies to clean up contaminated groundwater.

Established in 1995, GWRTAC is operated by Concurrent Technologies Corporation (CTC) in association with the University of Pittsburgh's Environmental Engineering Program through a Cooperative Agreement with the U.S. Environmental Protection Agency's (EPA) Technology Innovation Office (TIO).

### **About "O" Series Reports**

This report is one of the GWRTAC "O" Series of reports developed to provide a general overview and introduction to a ground-water remediation technology. These overview reports are intended to provide a basic orientation to the technology. They contain information gathered from a range of select, currently available sources, including project documents, reports, periodicals, internet searches, and personal communication with involved parties. No attempts are made to independently confirm or peer review the resources used.

### **Disclaimer**

GWRTAC makes no warranties, express or implied, including without limitation, warranty for completeness, accuracy, or usefulness of the information, warranties as to the merchantability, or fitness for a particular purpose. Moreover, the listing of any technology, corporation, company, person, or facility in this report does not constitute endorsement, approval, or recommendation by GWRTAC, CTC, the University of Pittsburgh, or the EPA.

---

## ABSTRACT

This technology summary report is an overview of information collected by GWRTAC on *in situ* bioremediation as an *in situ* ground-water remediation technology. Information provided includes an introduction to the general principles and techniques, a discussion of the general applicability of the technology, and reported advantages and limitations of the technology. References are also provided.

Bioremediation is a managed or spontaneous process in which microbiological processes are used to degrade or transform contaminants to less toxic or nontoxic forms, thereby mitigating or eliminating environmental contamination. In the presence of sufficient oxygen (aerobic conditions) and nutrients, microorganisms will ultimately convert many organic contaminants to carbon dioxide, water and microbial cell mass. In the absence of oxygen (anaerobic conditions), the electron acceptor is a compound other than molecular oxygen, such as sulfate, nitrate, or carbon dioxide, and the contaminants will be ultimately metabolized to methane, limited amounts of carbon dioxide and a trace amount of hydrogen gas. To date, most bioremediation technologies deal with treatment of a wide variety of natural organic compounds, although many of the more persistent contaminants in the environment, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), are resistant to microbial remediation.

A number of environmental conditions can slow down or stop the biodegradation process. It is therefore important to complete a bioremediation feasibility investigation prior to making a decision to implement bioremediation at a site. Some of the parameters important to consider in site characterization include the biodegradability, phase-distribution, leaching potential, and chemical reactivity of the contaminants, soil type and properties, competition for oxygen, and the presence or absence of inhibitory substances. While bioremediation is advantageous in many ways, such as it ideally resulting in transformation of toxic compounds into harmless substances, its limitations and criteria for its use must be well understood. Some of the practical limitations of bioremediation include the degree of cleanup achievable, the timeframe over which the cleanup can be achieved, and the potential in some cases, to produce toxic byproducts. Costs for implementing a bioremediation system vary widely, depending upon the type and degree of enhancement needed, as well as other site-specific factors. Some broad ranges of costs which may apply, and factors which may influence cost, are presented herein.

Whether aerobic or anaerobic bioremediation processes may apply to a given site, natural attenuation versus enhanced *in situ* bioremediation or bioaugmentation may be among general options for consideration. These are briefly defined and discussed in this report. Types of enhanced *in situ* bioremediation further discussed include bioventing, air sparging/biosparging, liquid delivery systems, alternate electron acceptors (anaerobic bioremediation), and phytoremediation. Lastly, two commercially available products to enhance aerobic bioremediation, oxygen release compound (ORC), and oxygen microbubbles, are briefly discussed.

This document was prepared for distribution by the Ground-Water Remediation Technologies Analysis Center (GWRTAC). GWRTAC is operated by Concurrent Technologies Corporation (CTC) in association with the University of Pittsburgh's Environmental Engineering Program, under a Cooperative Agreement with the U.S. EPA's Technology Innovation Office (TIO).

---

## TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
<b>1.0 INTRODUCTION/REPORT ORGANIZATION .....</b>	<b>1</b>
<b>2.0 APPLICABILITY .....</b>	<b>3</b>
2.1 Contaminants.....	3
2.2 Site Conditions.....	3
<b>3.0 METHODOLOGY .....</b>	<b>5</b>
<b>4.0 LIMITATIONS.....</b>	<b>6</b>
<b>5.0 ADVANTAGES.....</b>	<b>8</b>
<b>6.0 COSTS .....</b>	<b>9</b>
<b>7.0 NATURAL ATTENUATION .....</b>	<b>10</b>
<b>8.0 ENHANCED <i>IN SITU</i> BIOREMEDIATION.....</b>	<b>11</b>
<b>9.0 BIOAUGMENTATION .....</b>	<b>12</b>
<b>10.0 ENHANCED <i>IN SITU</i> BIOREMEDIATION TECHNOLOGIES .....</b>	<b>13</b>
10.1 Bioventing .....	13
10.2 Air Sparging/Biosparging .....	13
10.3 Liquid Delivery Systems.....	13
10.4 Alternate Electron Acceptors - Anaerobic Bioremediation .....	14
10.5 Phytoremediation .....	14
<b>11.0 AEROBIC BIOREMEDIATION PRODUCTS .....</b>	<b>16</b>
11.1 Oxygen Release Compound (ORC).....	16
11.2 Oxygen Microbubbles .....	17
<b>12.0 REFERENCES CITED.....</b>	<b>18</b>
<b>13.0 ADDITIONAL REFERENCES .....</b>	<b>20</b>

---

## 1.0 INTRODUCTION/REPORT ORGANIZATION

Bioremediation is a managed or spontaneous process in which microbiological processes are used to degrade or transform contaminants to less toxic or nontoxic forms, thereby mitigating or eliminating environmental contamination. Microorganisms depend on nutrients and carbon to provide the energy needed for their growth and survival. Degradation of natural substances in soils and sediments provides the necessary food for the development of microbial populations in these media. Bioremediation technologies harness the natural processes by selecting or promoting the enzymatic products and microbial growth necessary to convert the target contamination to nontoxic end products (1).

In the presence of sufficient oxygen (aerobic conditions) and nutrients, microorganisms will ultimately convert many organic contaminants to carbon dioxide, water and microbial cell mass. In the absence of oxygen (anaerobic conditions), the electron acceptor is a compound other than molecular oxygen, such as sulfate, nitrate, or carbon dioxide, and the contaminants will be ultimately metabolized to methane, limited amounts of carbon dioxide and a trace amount of hydrogen gas. Oxygen, if present, is the electron acceptor used preferentially, as it provides maximum energy yield to the microorganisms, maximizing cell production and microorganism growth, per unit amount of electron donor used. Thus, aerobic bioremediation generally proceeds more quickly and provides a more complete degradation of the organic compounds than anaerobic bioremediation (2,3,4).

The objective of bioremediation is to immobilize contaminants or to transform them into chemical products no longer hazardous to the environment. Transformation and degradation processes differ depending on the physical environment, microbial communities, and contaminants. Intrinsic bioremediation (or natural attenuation or passive bioremediation) depends on indigenous micro flora to degrade contaminants using only nutrients and electron acceptors available *in situ*.

However, bioremediation will be less than optimal if the microorganisms' nutritional and physiological requirements are not met. Enhanced bioremediation technologies increase biodegradation rates by supplying those nutrients and electron acceptors, or control other factors that can be rate-limiting (e.g. temperature, pH). Typically, indigenous microbes are capable of affecting transformation because they are acclimated to the contaminant as well as their micro niche. However, research is underway at a number of facilities using exogenous, specialized microorganisms or genetically engineered microbes (GEMs) to optimize bioremediation. This approach, referred to as bioaugmentation, may be appropriate for ex situ bioremediation projects, but has yet to be proven effective *in situ* (5).

Over the last decade, progress has been made in expanding the number and type of contaminants to which bioremediation can be applied. Research has contributed greatly to understanding the biotic, chemical, and hydrologic parameters which contribute to or restrict the application of *in situ* bioremediation. Significant progress has also been made in the number of practical methods for implementing enhanced *in situ* bioremediation. For example, alternative strategies have been developed for delivering of chemical additives, such as oxygen. Chemical additives for increasing the bioavailability of recalcitrant organics have been identified. Techniques such as hydro fracturing have been developed for improved delivery of nutrients or microorganisms in low permeability geologic media. In addition, methods have been developed for creating passive treatment systems such as biofilters. These advances are important steps toward establishing bioremediation as one of the viable solutions for *in situ* remediation of contaminant mixtures in a wide range of complex environments. Bioremediation systems must be designed based upon a complete understanding of the biological process upon which their success depends. The extent of biodegradation, potential for intermediate metabolite accumulation, and nutrient and electron acceptor requirements must be evaluated to design a successful bioremediation system. This requires a complete understanding of the applicable metabolic pathways. (4,6).

Biodegradation of organic compounds is common in nature, but can take many years. The key to improving the usefulness of bioremediation for cleaning contaminated sites is to determine how to accelerate the rate of biodegradation to detoxify the target compound in a finite time period (i.e., weeks or months rather than years). An exception is when it can be shown that natural biodegradation rates are

---

sufficient to degrade the contaminant of interest prior to any adverse environmental impacts, in which case intrinsic bioremediation, with no enhancements, is acceptable.

This report will discuss *in situ* bioremediation technologies, first by presenting general information on applicability, methodology, advantages, limitations and cost of *in situ* bioremediation. Subsequent sections briefly discuss natural attenuation, enhanced *in situ* bioremediation, and bioaugmentation. Enhanced *in situ* bioremediation technology is then discussed in more detail, where an overview of several enhancement techniques is presented in subsections. Finally, a section is devoted to bioremediation products for oxygenation of groundwater to enhance aerobic bioremediation, and is followed by reference sections.

---

## 2.0 APPLICABILITY

### 2.1 Contaminants

To date, most bioremediation technologies deal with treatment of natural organic compounds. Contaminants that are readily biodegradable under aerobic conditions include:

- Petroleum hydrocarbons such as benzene, toluene, ethylene, and xylene (BTEX) and gasoline;
- creosote;
- volatile organic compounds (VOCs) such as trichloroethylene and perchloroethylene (TCE and PCE);
- pesticides;
- organic solvents; and,
- wood preservatives.

Pilot studies indicate the effectiveness of microbial remediation of nitrotoluenes in soils contaminated with explosives. Many of the more persistent contaminants in the environment, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), are resistant to microbial remediation, because of 1) the compound's toxicity to the organisms, 2) preferential feeding of microorganisms on other substrates, 3) the microorganism's lack of genetic capability to use the compound as a source of carbon and energy, or 4) unfavorable environmental conditions in the sediment for propagating the appropriate strain of microorganisms. Alteration of the environmental conditions can often stimulate development of appropriate microbial populations that can degrade the organic compounds. Such changes may include adjusting the concentration of the compound, pH, oxygen concentration, or temperature, or adding nutrients or microbes that have been acclimated to the compound (1). PCBs and PAHs can be transformed or partially metabolized as a second substrate. Though they might not be suitable for a growth substrate, they may be degraded by cometabolism, where microorganisms growing on one compound produce an enzyme which transforms another compound on which they can not grow. A variety of enzymatic reactions, such as oxidation, hydrolysis, reductive dehalogenation, catalyze the cometabolic processes (7). Methanotrophic bacteria (which degrade methane), in particular, produce enzymes which can initiate oxidation of a variety of carbon compounds.

While bioremediation cannot degrade inorganic contaminants (metals and radionuclides), it can be used to change the valence state of inorganics and cause adsorption, uptake, accumulation, and concentration of inorganics in micro- or macroorganisms. In some cases, the altered species will be more easily flushed from geological host material due to increased solubility. In other cases, the opposite will occur, and the contaminant will be immobilized *in situ*. These techniques, while still largely experimental, show considerable promise of stabilizing or removing inorganics from soils (2,6).

### 2.2 Site Conditions

A number of environmental conditions can slow down or stop the biodegradation process. For example (3, 4, 8):

- The concentration of the chemical from the waste or co-existing materials may be so high that it is toxic to the microorganisms;
- concentration gradients may be too steep to allow acclimation;
- the number or type of microorganisms may be inadequate for biodegradation, or the opportunity for colonization in the subsurface is inadequate;
- conditions may be too acid or too alkaline;
- the microorganisms may lack sufficient nutrients or enzymes (such as nitrogen, phosphorus, potassium, sulfur, or trace elements), which they need to use the chemical as a food source;
- mass transfer from pockets of NAPL-saturated soils or contaminants affiliated with low permeability lenses or layers may limit contaminant removal efficiency;

- 
- moisture conditions can be unfavorable (too wet or too dry); or,
  - the microorganisms may lack the oxygen, nitrate, or sulfate they need to use the chemical as an energy source.

Therefore, it is important to complete a bioremediation feasibility investigation. Characteristics that must be identified at a site include (2,4):

- The biodegradability of the contaminants;
- distribution of contaminant into soil, water, NAPL, and vapor phases;
- the leaching potential of the contaminants (e.g., water solubility and soil sorption coefficient);
- the chemical reactivity of the contaminants (e.g., tendencies toward nonbiological reactions, such as hydrolysis, oxidation and polymerization);
- depth and areal extent of the contaminants;
- soil type and properties (e.g., organic carbon content, mineral content, pH, porosity, permeability, bulk density, moisture content, nutrient level, water-holding capacity);
- the competition for oxygen (e.g., redox potential, ambient oxygen levels);
- the presence or absence of substances that are toxic to microorganisms; and,
- the ability of microorganisms in the soil to degrade contaminants.

Characteristics of the ideal candidate sites for successful implementation of *in situ* bioremediation include (8):

- A homogeneous and permeable aquifer;
- a contaminant originating from a single source;
- a low ground-water gradient;
- no soil contamination or free product; and,
- an easily degraded, extracted, or an immobilized contaminant.

Although most sites will not meet all of these criteria, bioremediation strategies may be developed for the non-ideal site. The bioremediation strategy may be developed with site-specific geological and microbiological data, combined with knowledge concerning the chemical, physical, and biochemical fate of the contaminants present.

---

### 3.0 METHODOLOGY

Bioremediation is a treatment process in which indigenous or inoculated microorganisms degrade (metabolize) hazardous substances into less toxic or nontoxic substances, by creating a favorable environment for those microorganisms. Bioremediation of organic compounds is a complex process, and its application to specific compounds is based on an understanding of the microbiology, biochemistry, genetics, metabolic processes, structure, and function of natural microbial communities. Microbiology must be combined with engineering to develop effective bioremediation processes.

The *in situ* bioremediation technology process consists of the following activities (8).

1. A site investigation to determine the transport and fate characteristics of organic waste constituents in the contaminated site.
2. Performance of treatability studies (using batch or flow-through microcosms) to determine the potential for bioremediation and to define required operating and management practices.
3. Removal of the source of the contaminant and recovery of free products.
4. Design and implementation of a bioremediation plan based on fundamental engineering principles.
5. Establishment of a monitoring program to evaluate performance of the remediation effort.

---

## 4.0 LIMITATIONS

*In situ* bioremediation in the saturated zone may fail due to the lack of adequate mass transport of the electron acceptor (usually oxygen). Site setting factors, such as average permeability, and the scale and degree of heterogeneity are the main factors governing the advective and diffusional transport rates in the subsurface. If transport rates are too low, *in situ* bioremediation of saturated zones is not a viable option (9). In general, bioremediation processes are more difficult to apply to clayey and other low-permeability soils.

As previously indicated, oxygen is the preferred electron acceptor, and is necessary for aerobic biodegradation. In the aqueous phase, residual concentrations of oxygen >1.0 mg/L should be monitored to ensure oxygen levels are not rate-limiting. In the gaseous phase, residual oxygen levels should be maintained at >2 to 4% (by volume) to ensure sufficient oxygen is present for effective aerobic bioremediation of organic contaminants (4).

There are also microbiologically-specific reasons for potential failure or poor performance of *in situ* bioremediation projects. Indigenous bacteria in some cases, may not contain the genetic information needed to produce the enzymes required to degrade the contaminant of interest. If an active indigenous microorganism population is not present at a site, presence of inhibitory or toxic conditions should be suspected, and alternatives to bioremediation considered (4). Unavailability of the hydrocarbon to the microorganisms may itself inhibit degradation. Published laboratory and field evidence has not clarified whether bacteria can degrade absorbed, or low solubility hydrocarbons, or whether the hydrocarbon must first be solubilized.

For major nutrients nitrogen and phosphorous, a typical C:N:P ratio of 100:10:1 on a weight basis should ensure adequate levels of N and P are present for bioremediation. The pH should generally fall between 5.5 to 8.5 S.U. for optimum efficiency of bioremediation. This may be a key indicator of soil bioremediation feasibility when soil-based systems are considered. In soil-based systems, soil water should be 25 to 85% of field capacity (optimally 75% and higher), to sustain microbial activity. The soil water allows transfer of the contaminant from product or solid phases to the microorganisms (4).

High concentrations of heavy metals, highly chlorinated organics, long chain hydrocarbons, or inorganic salts are likely to be toxic to microorganisms (2), causing reduced efficiency or failure of *in situ* bioremediation. In other situations, presence of significant quantities of materials more amenable to biodegradation than the target contaminant may render *in situ* bioremediation ineffective for the needed remediation.

Growth of biomass that may not take part in the biodegradation process may cause harm through biofouling and plugging of injection wells, galleries or surrounding formations, thus decreasing critical subsurface mass transport capabilities. Also, gaseous oxygen, and hydrogen peroxide that decomposes quickly to gaseous oxygen, leads to formation of large air bubbles that can block pores in the aquifer material and hence inhibit aquifer flows.

The degree of clean up possible by using bioremediation also has practical limits. Hydrocarbons at the low ppm level may not be capable of supporting significant levels of microbial activity even under stimulation. Sites with relatively high levels of hydrocarbon impact may actually be better candidates for bioremediation than those lightly impacted some levels slightly above regulatory action levels (9). *In situ* processes generally require longer time periods, often years, depending mainly on the degradation rates of specific contaminants, site characteristics and climate. Finally, production of by-products more toxic than the contaminant (e.g., TCE to vinyl chloride) may occur, requiring control techniques, such as additional monitoring, or ground-water extraction (15).

There are some specific limitations when hydrogen peroxide is used as electron acceptor to enhance the bioremediation, including the following.

1. Concentrations of H<sub>2</sub>O<sub>2</sub> greater than 100 to 200 ppm in groundwater are inhibiting to microorganisms.

- 
2. Microbial enzymes and high iron content of subsurface materials can rapidly reduce concentrations of hydrogen peroxide and reduce the zone of influence. Amended hydrogen peroxide can be consumed very rapidly near the injection well, which may limit 1) biological growth to the region near the injection well, limiting adequate contamination/microorganisms contact and 2) cause biofouling of wells which can retard the input of nutrients.

---

## 5.0 ADVANTAGES

*In situ* bioremediation technology harnesses naturally-occurring biogeochemical processes. It immobilizes or destroys, partially or completely, contaminants rather than transfer them from one environmental media to another. Since the waste is treated on site, one has no future liability associated with the hauling and disposal of the waste on site. This treatment method has the potential of reducing further spread of contamination. The increased number of microorganisms will reduce the soil permeability, and therefore, reduce the rate of migration of contaminants (5,10).

*In situ* bioremediation can often be used to treat contaminants that are sorbed to aquifer materials or trapped in pore spaces. The areal zone of treatment can be larger than with other remedial technologies because the treatment moves with the plume and can reach areas that could otherwise be inaccessible.

---

## 6.0 COSTS

Factors that influence the capital and operating costs of bioremediation include:

- The type and quantity of organic compounds present;
- site conditions;
- the volume of material to be processed;
- the site-specific remediation goal;
- the nature and depth of the contaminants; and,
- the use of bioaugmentation and/or hydrogen peroxide or other enhancement additives.

There are too many variables in design and operational needs to give accurate ranges of costs. Nevertheless, typical costs for *in situ* bioremediation range from \$30 to \$100 per cubic meter (\$20 to \$80 per cubic yard) of soil. Contaminated groundwater and soil can be treated simultaneously, providing cost advantages. When hydrogen peroxide is used to enhance bioremediation, typical costs are \$10 to \$20 per 1,000 liters (\$50 to \$100 per 1,000 gallons) of groundwater treated. Operation and maintenance costs can be significant because a continuous source of hydrogen peroxide must be delivered to the contaminated groundwater (2,3).

---

## 7.0 NATURAL ATTENUATION

Natural attenuation occurs when physical, chemical and biological processes act to reduce the toxicity and mobility of subsurface contamination. The predominant processes that comprise natural attenuation include (11,12):

- **Biodegradation:** Indigenous microorganisms degrade many organic compounds by oxidation-reduction reactions.
- **Chemical Transformation:** Contaminants are altered and attenuated by subsurface chemical reactions such as hydrolysis and dehalogenation. The rate of chemical transformation depends on several variables, including soil and groundwater pH, temperature and chemical composition.
- **Stabilization:** In this process, contaminants become chemically bound by a stabilizing agent (e.g. clay, humic materials) and are prevented from migration.
- **Volatilization:** Volatilization may contribute to natural attenuation through transfer of VOCs from groundwater to the vadose zone or to the atmosphere, but it is comparatively a minor component of natural attenuation.
- **Dispersion and Dilution:** Assuming that the source of contamination is not ongoing, as a plume moves vertically and laterally from the release area, the ratio of groundwater to contamination increases and contaminant concentration decreases.

Responsibly proposed natural attenuation at a ground-water remediation site requires demonstration that one or more of these processes will occur at a sufficient rate to remediate the plume prior to impact at a receptor. Therefore, a site assessment must be completed, identifying the following:

1. Soil characteristics such as soil type, hydraulic conductivity and organic content.
2. Groundwater characteristics such as direction, gradient and velocity of flow, temperature, pH and dissolved oxygen content inside and outside the plume.
3. Horizontal and vertical extent of contamination in soil and groundwater, historical behavior of the plume, and the ability for attenuation of the plume under site conditions.
4. A detailed review of receptors and migration pathways in the area surrounding the site.

Once the site is assessed and the contaminant source is removed, a model should be developed to predict time, distance and direction of plume travel prior to degradation.

Since natural attenuation is a naturally occurring phenomenon, it requires little active or costly remedial action other than monitoring the natural process. There are three primary methods to quantify the extent of this passive bioremediation: 1) compound disappearance, 2) reduction of oxygen, nitrate, and sulfate concentrations relative to background concentrations, and 3) degradation byproducts such as increase of carbon dioxide (13).

With considerable cost savings compared to active remediation, natural attenuation allows resources to be devoted to higher priority sites. However, use of natural attenuation may impact property transfers because longer time frames are necessary to reach remedial objectives. Also, more extensive site characterization and monitoring may be required compared to conventional remedies due to the liability concerns caused by the potential for plume migration beyond property boundaries or to sensitive receptors (14).

---

## 8.0 ENHANCED *IN SITU* BIOREMEDIATION

When the rate of natural bioremediation is limited by nutrient and electron acceptor availability, enhanced *in situ* bioremediation may be applied. This technology is directed towards stimulating the microorganisms to grow and use the contaminants as a food and energy source by creating a favorable environment for the microorganisms. Generally, this means providing some combination of oxygen, nutrients and moisture, and controlling the pH and temperature (3,15).

To ensure that oxygen is supplied at a rate sufficient to maintain aerobic conditions, forced air, liquid oxygen, hydrogen peroxide, or ozone injection can be used. A study conducted by researchers at Utah State University utilized a prepared bed treatment unit for soil contaminated with PAHs and pentachlorophenol (PCP). The goal was to determine effects of oxygen concentration in the soil gas on biodegradation rates. It was possible to measure only biodegradation in relation to a decrease in oxygen by measuring mineralization as an indicator of biodegradation. The study demonstrated that significant mineralization occurred once oxygen concentration was available at a concentration of 2 to 5% in soil gas. Additional enrichments of the soil with oxygen did not produce a significant increase in mineralization rates. The researchers confirmed that there is a relationship between the concentration of oxygen in the soil gas and the rate of PAH and PCP disappearance.

Nutrients required for cell growth are nitrogen, phosphorus, potassium, sulfur, magnesium, calcium, manganese, iron, zinc, and copper. If nutrients are not available in sufficient amounts, microbial activity will stop. Nitrogen and phosphorus are the nutrients most likely to be deficient in the contaminated environment, and thus are usually added to the bioremediation system in a useable form (e.g., as ammonium and as phosphate).

The pH affects the solubility, and consequently the availability of many constituents of the soil, which can affect biological activity. Many metals that are potentially toxic to microorganisms are insoluble at elevated pH; therefore, elevating the pH of the treatment system can reduce the risk of poisoning the microorganisms.

Microbial activity in the environment is greatly affected by temperature, slowing with decreasing temperature. In northern climates bioremediation may be carried out in a climate-controlled facility to temper the seasonal variations. Microorganisms remain viable, but dormant, at temperatures below freezing and then resume activity when the temperature rises. Provisions for heating the bioremediation site, such as warm air injection, may speed up the remediation process. Temperature also affects non-biological losses of contaminants mainly through evaporation of contaminants at high temperature. The solubility of contaminants typically increases with increasing temperature. However, some hydrocarbons are more soluble at low temperatures than at high temperatures. Additionally, oxygen solubility decreases with increasing temperature.

Enhanced *in situ* bioremediation of soil typically involves the percolation or injection of groundwater or uncontaminated water mixed with nutrients and saturated dissolved oxygen, using injection and extraction wells. Placement of the wells is dependent on the area of contamination and the permeability of the formation, but are usually not more than 30 meters apart. Sometimes acclimated microorganisms (bioaugmentation) and/or other another oxygen source such as hydrogen peroxide are also added. An infiltration gallery or spray irrigation is typically used for shallow contaminated soils, and injection wells are used for deeper contaminated soils (2).

---

## 9.0 BIOAUGMENTATION

Bioaugmentation involves the addition of microorganisms with specific metabolic capabilities to a contaminated site, in addition to stimulating indigenous microbial populations to degrade organic contaminants. Populations that are specialized in degrading specific compounds are selected by enrichment culturing or genetic manipulation. Enrichment culturing involves exposure of microorganisms to increasing concentrations of a contaminant or mixture of contaminants.

However, the inoculation of a specialized microbial population into the environment may not produce the desired degree of degradation for a number of reasons. Factors that may limit the success of inoculants include contaminant concentration, pH, temperature, salinity, and osmotic and hydrostatic pressure. They may act alone or collectively to inhibit the survival of the microorganisms. The subsurface environment may also contain substances or other organisms that are toxic or inhibitory to the growth and activity of the inoculated organisms. In addition, adequate mixing to ensure contact of the organism with the specific organic constituent may be difficult to achieve at many sites. Successful inoculation of introduced organisms into simpler, more controllable environments (e.g., bioreactors) to accomplish degradation has been demonstrated. However, effectiveness of inoculation into uncontrolled environments such as the subsurface is much more difficult to achieve, demonstrate, and assess (8).

---

## 10.0 ENHANCED *IN SITU* BIOREMEDIATION TECHNOLOGIES

### 10.1 Bioventing

The bioventing biological system treats contaminated soil *in situ* by injecting atmospheric air into unsaturated soil. The air provides a continuous oxygen source, which enhances the growth of microorganisms naturally present in the soil. Additional additives such as ozone or nutrients may also be used to stimulate microbial growth. Bioventing accelerates the natural processes. The focus is on minimizing hydrocarbon volatilization (7, 16,17).

Bioventing technology uses an air pump attached to one of a series of air injection probes. The air pump operates at extremely low pressures, providing inflow oxygen without significantly volatilizing soil contaminants. The treatment capacity depends on the number of injection probes, the size of the pumps and the site characteristics such as soil porosity. Some systems use air injection wells in conjunction with air recovery wells.

Bioventing is typically used to treat contaminated soil and removes and/or degrades a number of contaminants that are biodegradable under aerobic conditions. Bioventing treats contaminants and combinations of contaminants with varying degrees of success. Volatile, nonbiodegradable constituents can be treated, but offgas treatment costs may be incurred or increased. Bioventing is most applicable where the depth to water exceeds three meters and the surficial soils do not require treatment or are being treated by another method. Shallower soils and sites with shallower water tables can be treated if the surface is capped.

Where applicable, bioventing has the potential to be a low cost remediation method. Because of the reduced need for offgas treatment and lower volumes of air moved through the soil, bioventing should be less costly than vapor stripping.

### 10.2 Air Sparging/Biosparging

Air sparging involves injecting a gas (usually air/oxygen) under pressure into the saturated zone to transfer volatile compounds to the unsaturated zone for biodegradation. The term biosparging is used to highlight the bioremediation aspect of the treatment process or refers to a situation where biodegradation is the dominant remedial process, with volatilizing playing a secondary role. The air injected below the water table increases the oxygen concentration and enhances the rate of biological degradation of organic contaminants by naturally occurring microorganisms. The addition of bioremediation processes makes application of air sparging more favorable for the remediation of less volatile contaminants like diesel fuel and waste oils (7,18,19).

Air injection flow rates must be carefully controlled to prevent transfer of volatile constituents to the atmosphere. Some form of vapor recovery, and possibly, ground-water protection may be necessary to prevent or limit losses of contaminants. Air sparging is less expensive, distributes oxygen across the site more quickly, and is associated with fewer operational problems than the liquid delivery method.

Further information about air sparging is available in the GWRTAC Technology Overview Report, TO-96-04, "Air Sparging", available at <http://www.gwrtac.org>.

### 10.3 Liquid Delivery Systems

Liquid delivery systems are used for bioremediation of contamination in the saturated zone. The delivery system, consisting of wells or trenches, is designed to circulate adequate amounts of nutrients and oxygen through the zone of contamination to maximize contaminant biodegradation. Groundwater is extracted, treated above ground if necessary, and then disposed or amended with nutrients and recirculated. The recirculation system is designed to hydraulically isolate the target area and minimize contaminant

---

migration out of the treatment zone. Oxygen is provided by sparging with air or pure oxygen or by adding hydrogen peroxide to injected water (7).

The cost of implementing a liquid delivery system depends on several factors, including the type, amount, and extent of contamination, sediment characteristics, and the source of oxygen. Liquid delivery may be best applied in situations where a pump-and-treat system is already in place or where site conditions, such as fractured rock aquifers, aquifers with shallow water tables, or formations with narrow saturated intervals, preclude air sparging. Also, if control of plume migration is mandated, liquid delivery may be advantageous (7).

## 10.4 Alternate Electron Acceptors - Anaerobic Bioremediation

Most bioremediation technologies have focused on the addition of oxygen to facilitate the biodegradation. Alternate electron acceptors can be used for bioremediation in the saturated zone. Because more energy is derived from aerobic respiration than other microbial processes, oxygen is the preferred electron acceptor, if present. But, because of the limited solubility of oxygen in water, it is difficult to deliver large quantities of dissolved oxygen to contaminated subsurface environments. A variety of oxy-anions can substitute for oxygen and allow microbial degradation of organic contaminants. Practical alternate acceptors include nitrate, sulfate, and salts of iron III. Various types of anaerobic bacteria exist in nature that can use these anaerobic electron acceptors (7,23).

Nitrate is much more soluble in water than oxygen, and is less reactive and more mobile. Nitrate also has a high electron-accepting capacity. Although nitrate is very soluble in water, the usual end product of nitrate reduction,  $N_2$ , is poorly water soluble. If  $N_2$  accumulates, bubbles may form that exclude water from the pore spaces and decrease the hydraulic conductivity of the subsurface material. Also, nitrate is expensive and toxic to humans.

Geovation has developed an *in situ* bioremediation technology -- Denitrification Based Bioremediation (DBB) -- that utilizes a combination of natural physical, geological and biogeochemical processes to achieve remediation of organic compounds, including petroleum hydrocarbons, halogenated solvents, and organo-pesticides. For the implementation of this technology, Geovation developed the Anaerobic Bioremediation Systems (ABS). More information about this technology is available at <http://www.geovation.com/dbbmain.htm> (20).

Sulfate is also highly soluble in water, has a high electron-accepting capacity for its mass, and does not sorb appreciably. It is inexpensive and not toxic for microorganisms. But, sulfide, the end product of sulfate reduction, is toxic to both humans and microorganisms.

Iron III salts are slightly soluble in water and have a low electron-accepting capacity for its mass. Practical applications may involve mechanically blending iron minerals or iron salts with contaminated material. Iron II, the end product of iron reduction, is not toxic at concentrations that would be expected during bioremediation.

## 10.5 Phytoremediation

Phytoremediation uses plants to clean up contaminated soil and groundwater, taking advantage of plants' natural abilities to take up, accumulate, and/or degrade constituents of their soil and water environments. Plant-assisted bioremediation, sometimes referred to as a type of phytoremediation, involves the interaction of plant roots and the microorganisms associated with root systems to remediate soils containing elevated concentrations of organic compounds. These techniques could provide cost-effective methods of remediating soil and groundwater contaminated with metals, radionuclides, and various types of organics, with less secondary waste and less environmental impact than would be generated using traditional remediation methods (21).

---

All plants extract necessary nutrients, including metals, from their soil and water environments. Some plants have the ability to store large amounts of metals, even some metals that do not appear to be required for plant functioning. In addition, plants can take up various organic chemicals from environmental media and degrade or otherwise process them for use in their physiological processes.

Phytoremediation technologies are in the early stages of development, with laboratory research and limited field trials being conducted to determine processes and refine methods. For more information consult the GWRTAC Technology Evaluation Report "Phytoremediation", TE-98-01, and the GWRTAC Technology Overview Report "Phytoremediation", TO-96-03, both available at <http://www.gwrtac.org>.

---

## 11.0 AEROBIC BIOREMEDIATION PRODUCTS

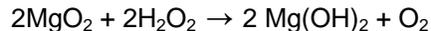
Various remediation technologies add oxygen and/or an oxygen source such as hydrogen peroxide, or ozone to groundwater or soil. Various products have been developed to oxygenate groundwater and/or soil. The following describes some of those products.

### 11.1 Oxygen Release Compound (ORC)

In February 1995, Regenesis Bioremediation Products, Inc., of San Juan Capistrano, California, introduced a new bioremediation product, called Oxygen Release Compound (ORC), to the environmental market, after three years of testing and development (22,23). Detailed information and numerous technical publications describing application of ORC are available at [www.regenesis.com](http://www.regenesis.com).

#### Product Description

Oxygen Release Compound (ORC) is a patented formulation of magnesium peroxide,  $MgO_2$ , which when moist, releases oxygen slowly. The hydrated product is magnesium hydroxide,  $Mg(OH)_2$ . The oxygen release rate is dependent upon the level of the contaminant flux. Generally, the product will continue to release oxygen for about six months, following the reaction:



The hydrated product is a harmless milk of magnesia which converts a solid form. This ORC residue may be used as a grout or filler material in the saturated zone.

ORC is a low solubility compound and thus releases dissolved oxygen slowly, for wide dispersal through the aquifer. This release mechanism minimizes iron fouling complications, and biofouling is inhibited by the elevated pH.

#### Applications

ORC may be used in the source area of the ground-water contaminant. For source treatment, ORC may be applied using retrievable filter socks placed in completed monitoring wells, or in a water and ORC powder slurry mixture. In this slurry form the ORC may be back filled or injected into direct-push bore holes, or back filled into augured holes. With these methods, a saturated zone source treatment with an ORC slurry targets dissolved phase contamination plus sorbed material in the saturated, capillary fringe and smear zones. It is important that the entire vertical distance of these contaminant zones be covered by the ORC for a source treatment.

Another application of ORC is the ORC oxygen barrier treatment. The objective is to reduce liability by stopping the migration of a contaminated ground-water plume beyond the property boundary or to achieve compliance at a point downgradient from the source. The ORC is best applied in completed monitoring wells with screened intervals through the contaminated portion of the saturated zone. In this application, a mixture of ORC and inert silica sand is contained in filter socks. After six months, when the oxygen is depleted, the socks may be removed from the wells. In order to achieve and maintain compliance, the ORC oxygen barrier must be recharged and the number of socks needed must be reevaluated. Depending upon the continuance of the contaminant source, if the load drops off, or increases, the number of socks may be decreased, or increased, accordingly.

For some contaminants, such as chlorinated hydrocarbons, there are dual-phase requirements for complete remediation to dechlorinated endpoints. In these cases the bioremediation process is initiated under anaerobic conditions and completed in the presence of oxygen. ORC can be used at the oxygen-dependent stages or to support various co-metabolic processes. When oxygen is needed to specifically inhibit certain reactions-such as the formation of vinyl chloride- ORC may be used as a preventative treatment.

---

### Costs

The costs of an ORC source treatment for groundwater could be 50% to 75% of that for traditional treatment systems such as pump and treat and air sparging. Following is the cost for an ORC treatment of a site in Oklahoma of 9,146 square feet, contaminated with BTEX (peak concentration in groundwater at 25 ppm). The ORC treatment is sized to close the site in one year.

System Installation: \$28,331  
Operation Costs: \$17,955  
**Total Costs: \$46,286**

## **11.2 Oxygen Microbubbles**

OHM Remediation Services Corporation (OHM) in Findlay, Ohio, has been developing techniques to deliver oxygen to the subsurface to enhance *in situ* microbial degradation, such as bioventing and biosparging techniques. They developed a product, oxygen microbubbles for *in situ* bioremediation (24). Further information is available at [www.clu-in.com/site/ongoing/emertech/ohm.htm](http://www.clu-in.com/site/ongoing/emertech/ohm.htm).

### Product Description

The microbubble process consists of generation of oxygen-rich microbubbles which are introduced into the subsurface to provide an oxygen source for degradation of contaminants. Oxygen microbubbles can be continuously generated by mixing water with a biodegradable surfactant at a concentration of approximately 200 mg/L. This solution is then mixed with a gas (air or oxygen) and is pumped to a continuous mixer. After passing through the mixer, a continuous flow of bubbles ranging in size from 45 to 100 microns in a dispersion contain 60% to 80% gas, is generated and can be injected into the subsurface by various methods.

The oxygen microbubbles tend to flow through into areas with high permeability, such as coarser zones. Contaminated groundwater flows through the treatment zone and bioremediated using the available oxygen. Indigenous microorganisms, often supplemented by introduced nutrients, provide the environment for *in situ* biodegradation of contaminants in groundwater or soil.

### Applications

OHM has conducted laboratory tests and field demonstrations of the oxygen microbubble technology in conjunction with the U.S. Environmental Protection Agency and the U.S. Air Force's Armstrong Laboratory.

A test was conducted to evaluate the microbubble injection characteristics as compared to air sparging techniques. The microbubbles exhibited different migration characteristics than typical sparged air. They did not develop channeling as did sparged air and were persistent in the pore space for longer than sparged air. These characteristics may make microbubbles an effective delivery system for a variety of contaminants not effectively treated by typical air sparging techniques. OHM is continuing the evaluation of this innovative technology application at sites exhibiting various hydrogeologic characteristics and contaminant types.

---

## 12.0 REFERENCES CITED

1. "Treatment Technologies", ARCS Remediation Guidance Document - Chapter 7, 24 pp., available at <http://epaserver.siesin.org:7777/gltreis/glnpo/data/arcs/EPA-905-B94-003/B94-003.ch7.html>.
2. "Biodegradation (In Situ)", Technologies Encyclopedia, 1996, available at <http://cluin.com/bioinsitu.htm>.
3. U.S. environmental Protection Agency, 1991, "Understanding Bioremediation; A Guidebook for Citizens", EPA/540/2-91/002, Research and Development, February 1991, 19pp.
4. Anderson, W.C., et al., Eds., 1998, Innovative Site Remediation Technology Design and Application; Vol. 1 - Bioremediation, American Academy of Environmental Engineers, WASTECH®.
5. "Bioremediation: A Single-Celled Solution for Reducing Environmental Contamination", About Biogroup, available at <http://biogroup.gzea.com/about.htm>.
6. "Biogeochemical Processes: The Foundation for In Situ Bioremediation", 1996, available at <http://bordeaux.uwaterloo.ca/biol447/new/DoERemediation/insitu.htm>.
7. Anderson, W.C., C.H. Ward, 1995, Innovative Site Remediation Technology; Vol. 1 - Bioremediation, American Academy of Environmental Engineers, WASTECH®.
8. Sims, J.L., J.M. Suflita, H.H. Russell, 1992, "Ground Water Issue: In Situ Bioremediation of Contaminated Ground Water", EPA/540/S-92/003, U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response, February 1992, 11pp.
9. Vance, D.B., 1993, "In Situ Bioremediation of Groundwater Understanding Limitations on the Process", 2 The 4 Technology Solutions, August 1993, 3pp., available at <http://www.flash.net/~nm2the4/insitubio.htm>.
10. U.S. Department of Energy, 1995, "In Situ Bioremediation of Groundwater", DOE, October 1995, 5pp, available at <http://www.em.doe.gov/rainpluj/plum321.html>.
11. Ritz, S.M., 1996, "States Speaks Out on Natural Attenuation", available at <http://www.gvi.net/soils/JanFeb96/statespeak.html>.
12. Remediation Technologies Development Forum, 1996, "Guidance Handbook on Natural Attenuation of Chlorinated Solvents", Bioremediation of Chlorinated Solvents Consortium, RTDF, September 1996, 46 pp.
13. Underground Storage Tank Cleanup Fund, Cost Guidelines, 1996, "Project Costs, Passive Bioremediation", California State Water Resources Control Board, USTCF, available at <http://www.sercb.ca.gov/cwphone/s2p9.htm>.
14. "Appendix C: Natural Attenuation Position Paper", Interstate Technology and Regulatory Corporation Work Group, available at <http://www.gnet.org/gnet/gov/interstate/appc.htm>.
15. "In Situ Biological Treatment for Groundwater, Surface Water, and Leachate", 1996, in Remediation Technologies Screening Matrix and Reference Guide, available at [http://em-50.em.doe.gov/BEST/Integration\\_Technologies/section3.8.html](http://em-50.em.doe.gov/BEST/Integration_Technologies/section3.8.html).
16. Buckeley, M., 1997, "Bioventing Takes Natural Approach to Cleaning Soil", available at <http://aec-www.apgea.army.mil:8080/prod/usaec/op/update/fall96/biovent.htm>.

- 
17. Risk Reduction Engineering Laboratory, 1994, "Bioventing", SITE Technology Profile, Demonstration Program, available at [www.gnet.org/gnet/tech/techb/site/demongng/rrel.htm](http://www.gnet.org/gnet/tech/techb/site/demongng/rrel.htm).
  18. "Oxygen Enhancement with Air Sparging", available at [http://pipes.ehsg.saic.com/section4/4\\_32.html](http://pipes.ehsg.saic.com/section4/4_32.html).
  19. Miller, R., 1996, "Technology Overview Report: Air Sparging", Ground-Water Remediation Technologies Analysis Center (GWRTAC), Pittsburgh, PA, October 1996, 10pp, available at <http://www.gwrtac.org>.
  20. Geovation Technologies, 1997, "Anaerobic Bioremediation Systems (ABS) and Denitrification-Based Bioremediation (DBB) Technology", Geovation, Hariman, New York, available at <http://www.geovation.com/dbbmain.htm>.
  21. Miller, R., 1996, "Technology Overview Report: Phytoremediation", Ground-Water Remediation Technologies Analysis Center (GWRTAC), Pittsburgh, PA, October 1996, 9 pp, available at <http://www.gwrtac.org>.
  22. Regenesi Bioremediation Products, 1996, "Oxygen Release Compound, ORC", available at <http://www.regenesis.com>.
  23. Oencrantz, J.E., J.G. Johnson, S.S. Koenigberg, 1995, "Enhanced Intrinsic Bioremediation of Dissolved Phase Hydrocarbons Using an Oxygen Releasing Compound: Quantitative Field Demonstration in Belen, New Mexico", *Journal of Ground Water Monitoring and Remediation*, December 1995.
  24. OHM Remediation Service Corporation, 1994, "Oxygen Microbubble In Situ Bioremediation", SITE Technology Profile, Demonstration Program, available at <http://www.gnet.org/gnet/tech/techdb/site/emrgongo/ohmcorp.htm>.

---

### 13.0 ADDITIONAL REFERENCES

Lee, M.D., J.M. Thomas, R.C. Borden, P.B. Dediect, J.T. Wilson, and C.H. Ward, 1988, "Biorestitution of Aquifers Contaminated with Organic Compounds", CRC Critical Review of Environment Controls, Vol. 18, pp. 29-89.

Sulfito, J.M., 1989, "Microbial Ecology and Pollutant Biodegradation in Subsurface Ecosystems", In: Transport and Fate of Contaminants in the Subsurface. EPA/625/4-98/019, Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, OK.

Thomas, J.M., and C.H. Ward, 1989, "In Situ Biorestitution of Organic Contaminants in the Subsurface", Environmental Science and Technology, Vol. 23, pp. 760-766.