United States
Environmental Protection
Agency

Land And
Emergency Management
5401R

EPA 510-B-17-003
October 2017
www.epa.gov/ust

How To Evaluate Alternative Cleanup Technologies For Underground Storage Tank Sites

A Guide For Corrective Action Plan Reviewers

Chapter XII

Enhanced Aerobic Bioremediation

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Chapter XII
Enhanced Aerobic Bioremediation

Overview

Enhanced aerobic bioremediation technologies are used to accelerate naturally occurring in-situ bioremediation of petroleum hydrocarbons, and some fuel oxygenates such as methyl tertiary-butyl ether (MTBE), by indigenous microorganisms in the subsurface. Enhanced aerobic bioremediation technologies include biosparging; bioventing[1]; use of oxygen releasing compounds; pure oxygen injection; hydrogen peroxide infiltration; and ozone injection[2]. These technologies work by providing a supplemental supply of oxygen to the subsurface, which becomes available to aerobic, hydrocarbon-degrading bacteria. The stoichiometric ratio of oxygen per hydrocarbon is 3 M O_2 per 1 mole of hydrocarbons. Oxygen is considered by many to be the primary growth-limiting factor for hydrocarbon-degrading bacteria, but it is normally depleted in zones that have been contaminated with hydrocarbons. By using these technologies, rates of biodegradation of petroleum hydrocarbons can be increased at least one, and sometimes several, orders of magnitude over naturally-occurring, non-stimulated rates.

Enhanced aerobic bioremediation technologies can be used to address contaminants in the unsaturated zone, the saturated zone, or both. Bioventing, for example, specifically targets petroleum hydrocarbon contaminants in the unsaturated zones and does not address contaminants in the capillary fringe or saturated zone. Most, but not all, enhanced aerobic bioremediation technologies primarily address petroleum hydrocarbons and some oxygenates that are dissolved in groundwater or are sorbed to soil particles in the saturated zone. The technologies are typically employed outside heavily contaminated source areas which will usually be addressed by more aggressive remedial approaches.

When used appropriately, enhanced aerobic bioremediation technologies are effective in reducing levels of petroleum contamination at leaking underground storage tank sites. Gasoline constituents dissolved in water are a likely target of enhanced aerobic bioremediations. Enhanced aerobic bioremediation technologies are most often used at sites with mid-weight petroleum products (e.g., diesel fuel,

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[1] For more information on Biosparging and Bioventing, see *How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers* (US EPA 510-R-04-002), Chapter III ("Bioventing") and Chapter VIII ("Biosparging").

[2] Other enhanced aerobic bioremediation technologies, including surfactant enhanced microbubble injection and permeable polymeric tubing oxygen diffusion, are not discussed in this chapter because of their limited use and experimental status.

readily and can be removed more rapidly using other technologies (e.g., air sparging or soil vapor extraction). However, if these lighter products are present, enhanced aerobic bioremediation technologies can also effectively reduce contaminant concentrations. Heavier petroleum products such as lubricating oils generally take longer to biodegrade than the lighter products, but enhanced aerobic bioremediation technologies may still be effective at sites contaminated with these products.

It is generally not practical to use enhanced aerobic bioremediation technologies to address free mobile product or petroleum contamination in low permeability soil (e.g., clay). Because enhanced aerobic bioremediation is a relatively slow cleanup approach, it is not recommended to address current or imminent excessive human health or environmental risks.

Exhibit XII-1 summarizes the general advantages and disadvantages of enhanced aerobic bioremediation technologies. Discussions of bioventing and biosparging, two other enhanced aerobic bioremediation technologies, are provided in *How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers* (US EPA 510-R-04-002, 2004), Chapter III ("Bioventing"), Chapter VIII ("Biosparging"), and Chapter X ("In-Situ Groundwater Bioremediation").

A brief description of several of the technologies is provided below.

Oxygen Releasing Compounds

Various enhanced aerobic bioremediation approaches rely on oxygen releasing compounds to remediate petroleum contamination. More commonly used oxygen releasing compounds include calcium and magnesium peroxides that are introduced to the saturated zone in solid or slurry phases. These peroxides release oxygen to the aquifer when hydrated by groundwater as the peroxides are ultimately converted to their respective hydroxides. Magnesium peroxide has been more commonly applied in field applications than calcium peroxide because of magnesium peroxide's lower solubility and, consequently, prolonged release of oxygen. Magnesium peroxide formulations placed in the saturated zone during a short-term injection event can release oxygen to groundwater over a four- to eight- month period. Significant quantities of magnesium peroxide are required based on stoichiometry and the fact that 90% of the weight of the compound is not oxygen. Oxygen amounting to approximately 10% of the weight of magnesium peroxide placed in the saturated zone is released to the aquifer over the active period.

Exhibit XII-1
Enhanced Aerobic Bioremediation
Primary Advantages and Disadvantages

Advantages

- Works with and enhances natural in-situ processes already at play (typically uses natural groundwater gradient, naturally occurring biodegradation)
- Destroys the petroleum contamination in place
- Produces no significant wastes (off-gases or fluid discharges)
- Can be a low-energy approach
- Is relatively inexpensive
- Complements more aggressive technologies (e.g., groundwater extraction) and less aggressive approaches (e.g., intrinsic remediation) that can be integrated into site remediation
- Causes minimal disturbance to site operations
- Has simple operation and monitoring requirements
- Is potentially more reliable than other, more active remedial technologies (e.g., groundwater extraction and treatment)
- Can be used in tandem with other remedial technologies that address small amounts of residual soil and groundwater contamination

Disadvantages

- May have longer remedial time frames than more aggressive approaches
- May not be able to reduce contaminants to background or very low concentrations
- Typically requires long-term monitoring of residual contamination in soil and groundwater
- May require permits for nutrient/oxygen injection
- May not be fully effective on all petroleum hydrocarbons and product additives (e.g., MTBE)
- Often must be accompanied by other technologies (e.g., product recovery) to address source areas
- May significantly alter aquifer geochemistry
- Can be misapplied to remediation at some sites if the conditions for use are not fully understood
- Oxygen supplied by enhanced aerobic bioremediation may be lost to chemical reactions in the subsurface which do not promote hydrocarbon contaminant oxidation and degradation.

Exhibit XII-2 compares the relative advantages and disadvantages of several different enhanced aerobic bioremediation technologies currently in use.

Exhibit XII-2
Enhanced Aerobic Bioremediation Technologies Comparative Matrix

	Oxygen Releasing Compounds	**Hydrogen Peroxide Infiltration**	**Pure Oxygen Injection**	**Ozone Injection**	**Biosparging**	**Bioventing**
Advantages						
No mechanical components required	X					
Minimal engineering design requirements	X					
Relatively low capital and operating costs	X					
Abiotic oxidation of contaminants contacting reagents		X		X		
Remediates contamination in unsaturated soils	X	X	X	X	X	X
Locally saturates groundwater with oxygen to further enhance biodegradation and oxygen distribution	X	X	X	X		
Can efficiently sustain widespread ambient (up to 8 mg/L) oxygen concentrations in groundwater					X	
Can efficiently sustain widespread ambient (up to ~21%) oxygen concentrations in unsaturated soils						X
Generally considered safe	X					X
Electricity/power source generally not required	X	X				

Exhibit XII-2
Enhanced Aerobic Bioremediation Technologies Comparative Matrix (continued)

	Oxygen Releasing Compounds	Hydrogen Peroxide Infiltration	Pure Oxygen Injection	Ozone Injection	Biosparging	Bioventing
Disadvantages						
Heavy reliance on groundwater advection, dispersion, and diffusion to distribute oxygen can limit treatment coverage and prolong remediation	X	X	X			
Increased risk of fugitive vapors entering building structures and utility conduits, particularly in absence of vapor recovery technology (e.g., soil vapor extraction)			X	X	X	
Does not target or treat saturated zone						X
On-site reactive chemical handling and storage required		X				
On-site gas production and delivery equipment (e.g., ozone generator) typically required			X	X		
Relatively few petroleum remediation projects completed using this technology		X	X	X		
May require reinjection permits	X	X			X	X
Radius of influence limited if using “socks”	X					
Zone of influence may be limited with compounds that are suspended in a well.	X					

Oxygen releasing compounds may be introduced into the saturated zone in several ways. The most common approaches include:

- Placing the compounds into drilled boreholes or other excavations (e.g., tank fields)
- Injecting a compound slurry into direct-push borings (e.g., Geoprobe)

- Mixing oxygen-releasing compounds directly with contaminated soil and then using the mixture as backfill or hauling it to a disposal site
- Suspending oxygen releasing compounds contained in “socks” in groundwater monitoring wells
- A combination of the above

Oxygen-releasing compounds may also be used to address source areas, entire plumes or plume tails (e.g., a treatment curtain aligned perpendicular to contaminant flow direction). Exhibit XII-3 provides a conceptual depiction of the deployment of oxygen releasing compounds to address a petroleum hydrocarbon plume. Many site-specific conditions must be considered before a remedial approach using this technology can be devised and implemented. One such site-specific concern is the proximity of drinking water supply wells to the treatment area and how the injected oxygen or other nutrients may affect these wells. Another concern is the limited zone of influence of oxygen releasing compounds when deployed in a well, which often provide increased oxygen levels only up to twice the diameter of the well. While the scope of this document does not allow a more in-depth discussion of this or other site-specific implementation, it is important to carefully consider site-specific issues (e.g., contaminant composition and behavior, site geology and hydrology) along with the conceptual information provided in this chapter.

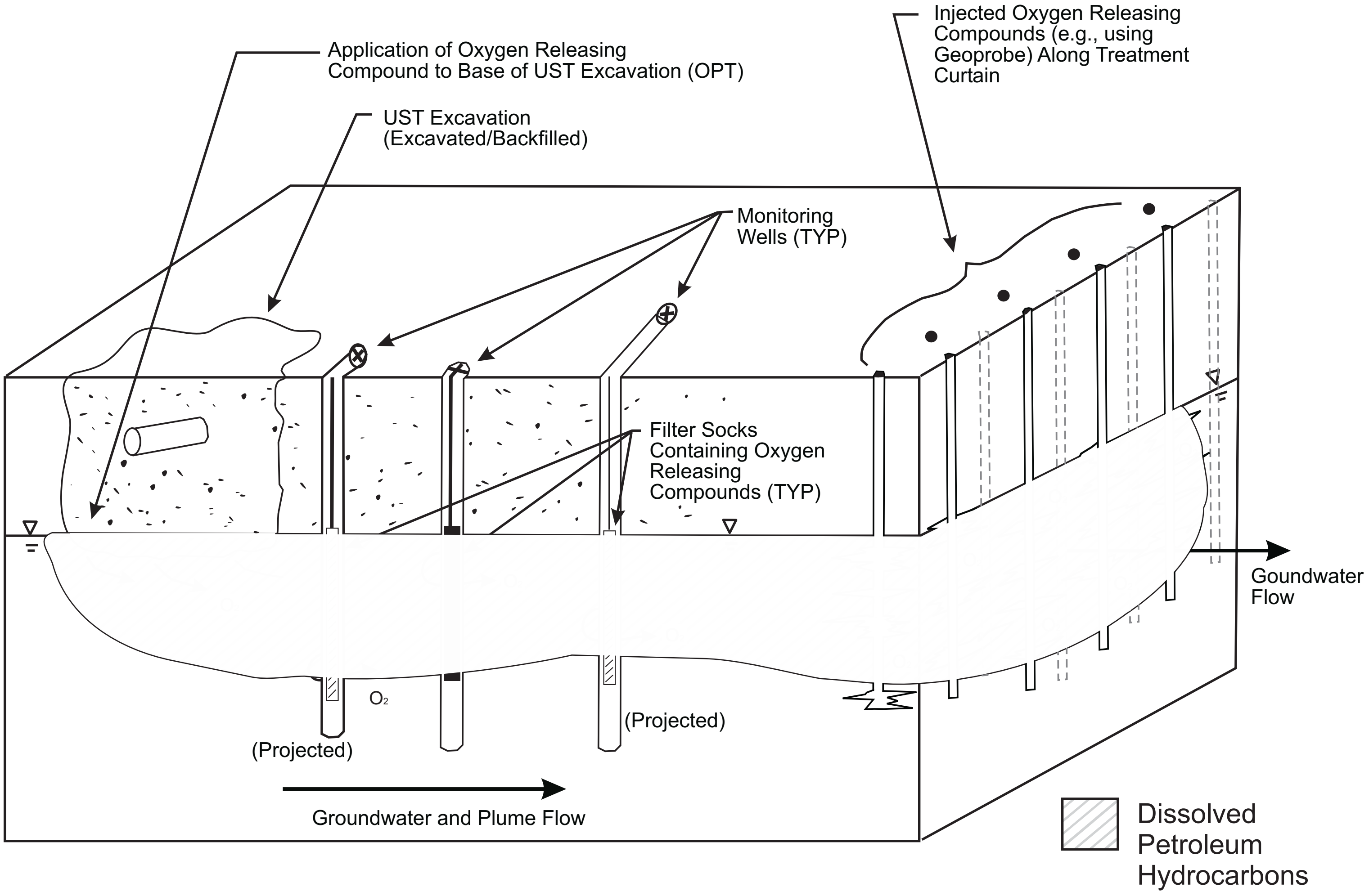
The following sections describe the use of pure oxygen injection, hydrogen peroxide infiltration, and ozone injection.

Pure Oxygen Injection

Injecting pure oxygen into groundwater can be a relatively efficient means of increasing dissolved oxygen levels in groundwater to promote aerobic biodegradation of petroleum hydrocarbons. In contrast to other enhanced aerobic bioremediation technologies, there is no carrier (e.g., amended groundwater) or delivery media (e.g., oxygen releasing compounds slurry) associated with pure oxygen injection. Approximately one gram of oxygen is delivered to the subsurface for every gram of oxygen directed to the subsurface. Oxygen is several times more soluble in groundwater when it is introduced in pure form than if the dissolved oxygen is derived by forcing groundwater to come into contact with atmospheric air, such as occurs with biosparging. Dissolved oxygen concentrations of up to 40-50 parts per million(ppm) can be achieved through pure oxygen injection, which contrasts to dissolved oxygen concentration limits of approximately 8-10 ppm when the saturated zone is aerated using atmospheric air, which contains approximately 21% oxygen.

Pure oxygen is most commonly introduced into the subsurface via vapor-phase injection. Vapor-phase oxygen (approximately 95% oxygen) is injected into the saturated zone near the base of the dissolved petroleum hydrocarbon contamination using a network of sparge wells. Oxygen sparge rates lower than

Exhibit XII-3
Typical Enhanced Aerobic Remediation Using Oxygen Releasing Compounds



air sparge flow rates are used in order to maximize contact time between the oxygen and contaminated groundwater before the injected oxygen rises through the contaminated zone to the water table. Trapping of sparged oxygen in the soil matrix (e.g., in soil pore spaces or semi-confining laminates) beneficially prolongs contact between the pure oxygen and the oxygen-depleted groundwater. Series of vertical oxygen injection wells are often alternately sparged in order to increase dissolved oxygen levels more efficiently over larger areas.

The spacing of injection wells is typically site-specific and based on the thickness of contaminated material, geology, hydrogeology, and other factors affecting the delivery and distribution of dissolved oxygen. Volatile organic vapor production and migration concerns are reduced with oxygen sparging relative to air sparging because of the significantly lower oxygen sparge air flow rates. However, vapor production and migration can be a concern and should be evaluated on a site-specific basis. A conceptual schematic of a pure oxygen injection system is depicted in Exhibit XII-4.

Hydrogen Peroxide Infiltration

Extracted and treated groundwater is amended and mixed with hydrogen peroxide prior to re-infiltration or re-injection. The hydrogen peroxide-amended groundwater is pumped into infiltration galleries or injection wells located in or near suspected source areas. Generally, the infiltration/injection and groundwater extraction scheme is designed to promote the circulation and distribution of hydrogen peroxide and dissolved oxygen through the treatment area.

Exhibit XII-5 provides a conceptual illustration of a hydrogen peroxide enhanced aerobic bioremediation system. The precipitation of chemical oxidants (e.g., iron oxides) can present potentially significant equipment fouling problems in this type of system, depending on the concentrations of naturally occurring levels of inorganic compounds, such as iron, in the subsurface.

Introducing hydrogen peroxide, which is a chemical oxidant, to the saturated zone can significantly augment existing oxygen levels because it naturally decomposes rapidly, generating oxygen. For each part (e.g., mole) of hydrogen peroxide introduced to groundwater, one-half part of oxygen can be produced. Hydrogen peroxide has the potential of providing some of the highest levels of available oxygen to contaminated groundwater relative to other enhanced aerobic bioremediation technologies because it is infinitely soluble in water. In theory, 10% hydrogen peroxide could provide 50,000 ppm of available oxygen.

However, when introduced to groundwater, hydrogen peroxide is unstable and can decompose to oxygen and water within four hours. This limits the extent to which the hydrogen peroxide may be distributed in the subsurface before it is transformed. Introducing concentrations of hydrogen peroxide as low as 100 ppm can cause oxygen concentrations in groundwater to exceed the solubility limit of

Exhibit XII-4
Typical Enhanced Aerobic Remediation Using Pure Oxygen Injection

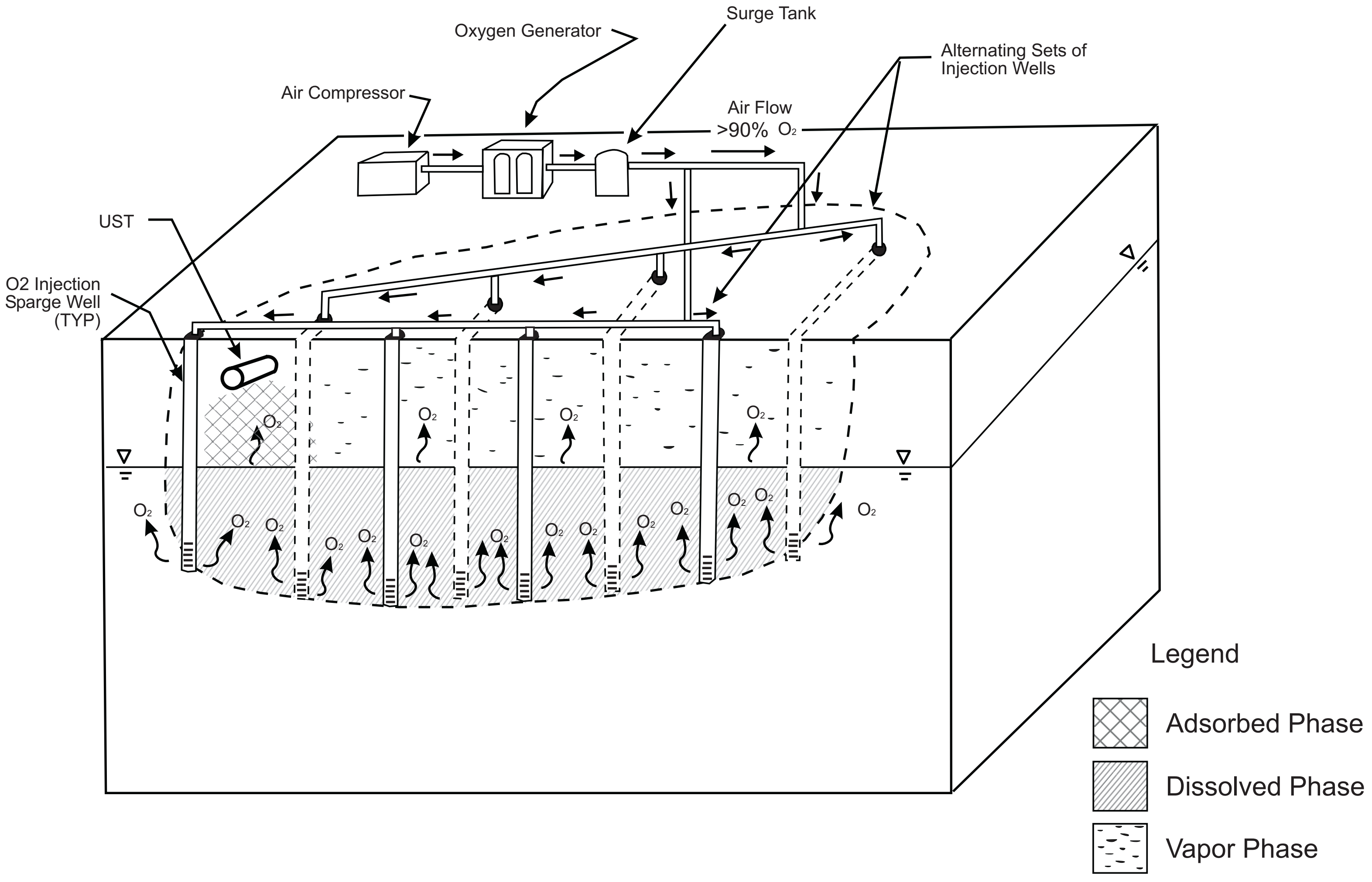
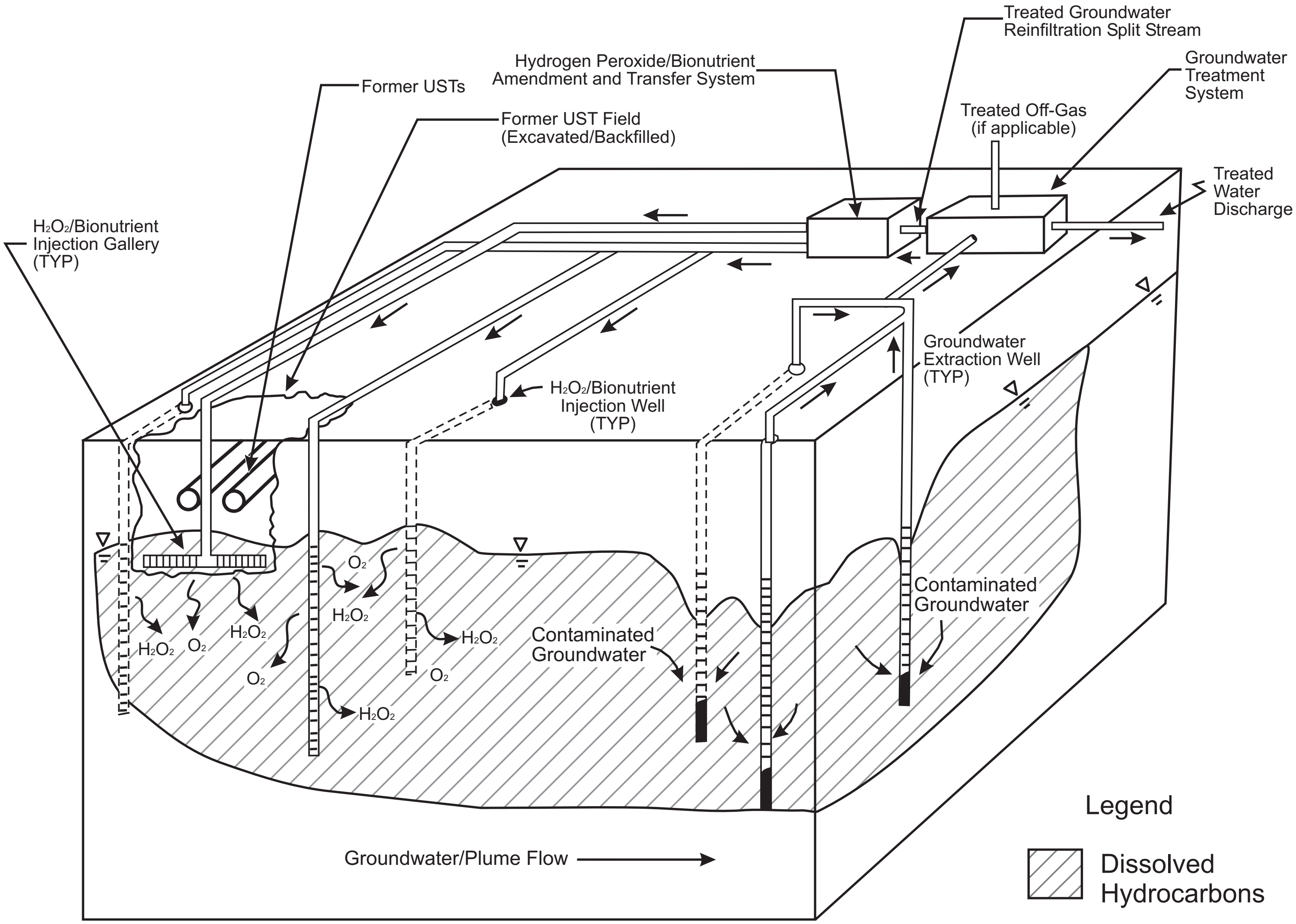


Exhibit XII-5
Typical Enhanced Aerobic Remediation Using Hydrogen Peroxide



oxygen in groundwater (40-50 ppm). When this occurs, oxygen gas is formed, which can be lost in the form of bubbles that rise through the saturated zone to the water table and into the unsaturated zone.

For enhanced aerobic bioremediation purposes, hydrogen peroxide is used at concentrations that maximize dissolved oxygen delivery to the petroleum-contaminated area while minimizing losses of oxygen through volatilization. Hydrogen peroxide is cytotoxic to microorganisms at concentrations greater than 100-200 ppm. This toxicity to aerobic petroleum degrading microbes can be amplified if carbon sources and nutrients are depleted in the contaminated media. Concentrations and application rates are typically determined on a site-specific basis, depending on site conditions, contaminant levels, and cleanup goals.

Hydrogen peroxide in a more concentrated form and in the presence of an iron catalyst can also be used to chemically oxidize site contaminants. This application of peroxide is not discussed in this chapter. When used in this manner, hydrogen peroxide's reaction with ferrous iron produces Fenton's reagent. Fenton's reagent chemical oxidation requires a comprehensive three-dimensional site characterization to locate preferential pathways for migration. It is important that any hydrogen peroxide remediation system contain an adequate number of soil vapor extraction wells to completely capture vapors. For more information on the use of hydrogen peroxide as an oxidant, see *How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers* (US EPA 510-R-04-002), Chapter XIII, “Chemical Oxidation”.

The potential dangers of working with hydrogen peroxide should not be overlooked when considering the technology and determining how it should be applied. Hydrogen peroxide is an oxidant that can cause chemical burns. When introduced into a petroleum-contaminated area at high concentrations, hydrogen peroxide can produce heat and elevated oxygen levels that may lead to fire or explosions. Use of concentrated peroxide should be avoided to help reduce these hazards.

Ozone Injection

Ozone injection is both a chemical oxidation technology and an enhanced aerobic bioremediation technology. Oxidation of organic matter and contaminants occurs in the immediate ozone application and decomposition area. Outside the decomposition area, increased levels of dissolved oxygen can enhance aerobic bioremediation. Ozone is a strong oxidant with an oxidation potential greater than that of hydrogen peroxide. It is also effective in delivering oxygen to enhance subsurface bioremediation of petroleum-impacted areas. Ozone is 10 times more soluble in water than is pure oxygen.

Consequently, groundwater becomes increasingly saturated with dissolved oxygen as unstable ozone molecules decompose into oxygen molecules. About one-half of dissolved ozone introduced into the subsurface degrades to oxygen within approximately 20 minutes. The dissolved oxygen can then be used as a source of energy by indigenous aerobic hydrocarbon-degrading bacteria.

Because of its oxidization potential, injected ozone can also be toxic to indigenous aerobic bacteria and can actually suppress subsurface biological activity. However, this suppression is temporary, and a sufficient number of bacteria survive in-situ ozonation to resume biodegradation after ozone has been applied.

Ozone may be injected into the subsurface in a dissolved phase or in gaseous phases. Groundwater is often extracted and treated, then used to transport (through re-injection or re-infiltration) the dissolved phase ozone and oxygen into the subsurface contaminated area. More commonly, however, gaseous ozone is injected or sparged directly into the contaminated groundwater. Because of its instability, ozone is generated on-site and in relatively close proximity to the target contaminated area. Typically, air containing up to 5% ozone is injected into strategically placed sparge wells. Ozone then dissolves in the groundwater, reacts with subsurface organics, and decomposes to oxygen. Vapor control equipment (e.g., an soil vapor extraction and treatment system) may be warranted when ozone injection rates are high enough to emit excess ozone to the unsaturated zone, which may slow deployment timetables in some states. In many states, vapor control equipment requires a permit for off-gas treatment.

Special Considerations for MTBE. The gasoline additive methyl tertiary butyl ether (MTBE) is often found in the subsurface when gasoline has been released. In addition, MTBE is sometimes discovered at spill sites of middle distillate petroleum products like diesel, jet fuel, kerosene, and fuel oil. As such, whenever a petroleum hydrocarbon spill is investigated and remediated, the presence/absence of MTBE in the soil and ground water should be verified.

Several crucial characteristics of MTBE affect the movement and remediation of MTBE, including:

- MTBE is more soluble in water than most C6-C10 gasoline-range hydrocarbons. For example, MTBE is 28 times more soluble in water than is benzene.

- MTBE is less volatile from water (i.e., has a lower Henry’s Constant) than most C6-C10 hydrocarbons. For example, MTBE is 11 times less volatile from water than is benzene.

- MTBE adheres less to soil organic matter than most C6-C10 hydrocarbons. This means that it has lower retardation and more rapid transport in groundwater than most gasoline-range compounds.

- At most sites, MTBE is less biodegradable in the subsurface than other gasoline compounds.

Because of these characteristics, some MTBE from a gasoline spill will be found with the BTEX compounds in the soil and groundwater near the site of petroleum release. But it is also quite common to find a dissolved-phase MTBE-only plume downgradient of the BTEX/TPH plume. Thus, when considering using enhanced aerobic bioremediation techniques for gasoline plumes that include MTBE, recognize that the MTBE may exist in two distinct regions:

- A near-source area where MTBE co-occurs with more readily biodegradable BTEX/TPH compounds
- A distal area where the only compound of concern is MTBE

Any petroleum impact remediation plan that addresses MTBE should account for the probable MTBE-only plume downgradient of the MTBE & hydrocarbon plume. The MTBE-only plume often has decreased levels of dissolved oxygen, due to its occurrence in the “oxygen shadow” region downgradient from the spilled petroleum source area where natural biodegradation is typically occurring (Davidson, 1995).

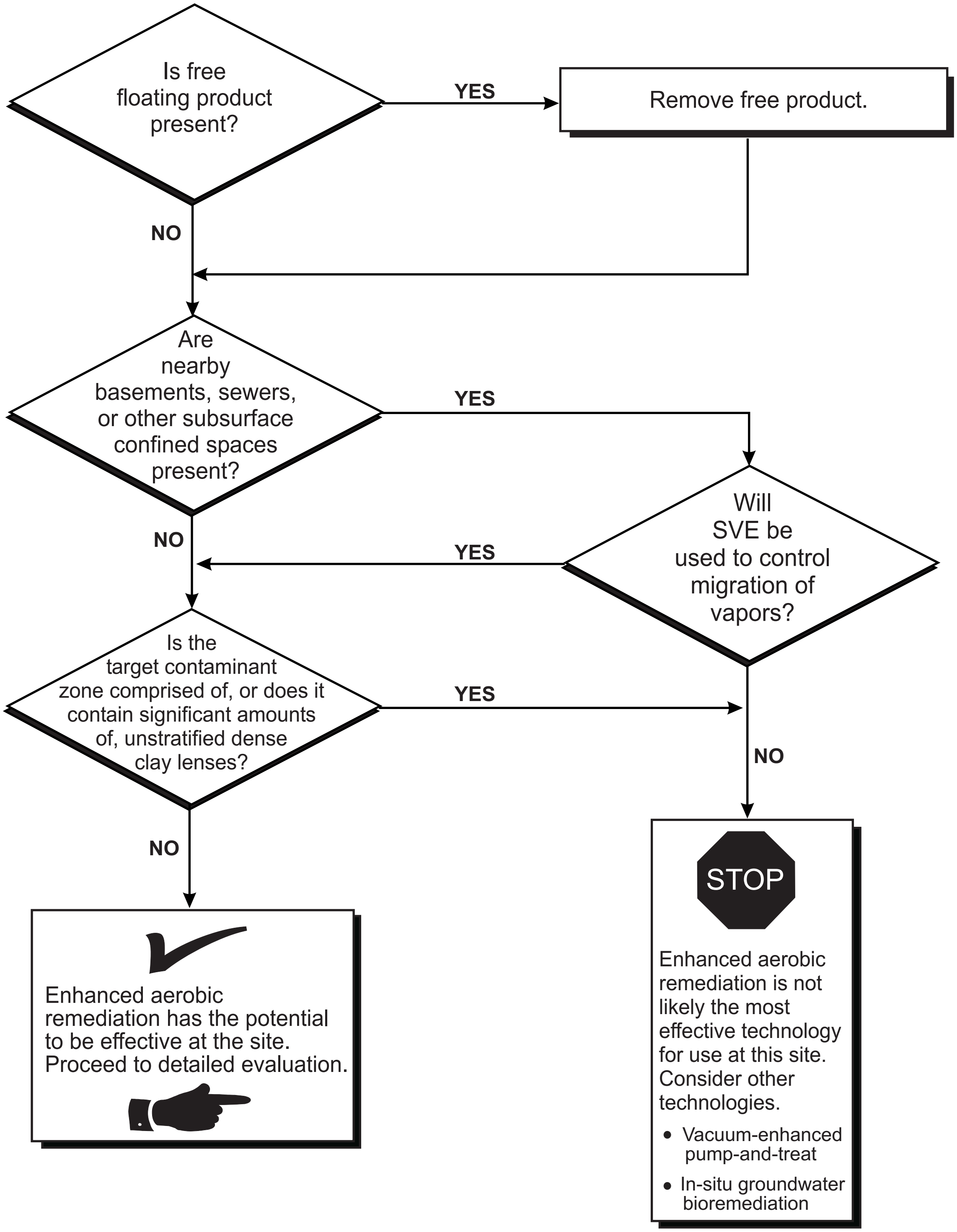
Enhanced Aerobic Bioremediation Technology Effectiveness Screening Approach

The descriptions of the various enhanced aerobic bioremediation technologies in the overview provide the basic information needed to evaluate a corrective action plan that proposes enhanced aerobic bioremediation. To assist with evaluation of the enhanced aerobic bioremediation corrective action plan, a step-by-step technology effectiveness screening approach is provided in a flow diagram in Exhibit XII–6. This exhibit summarizes this evaluation process and serves as a roadmap for the decisions to make during evaluation of the corrective action plan. A checklist has also been provided at the end of this chapter, which can be used to evaluate the completeness of the enhanced aerobic bioremediation corrective action plan and to focus attention on areas where additional information may be needed. The evaluation process can be divided into the four steps described below.

- **Step 1:** ***An initial screening of enhanced aerobic bioremediation effectiveness*** allows quick determination of whether enhanced aerobic bioremediation should be considered as a remedial approach for the site.

- **Step 2:** ***A detailed evaluation of enhanced aerobic bioremediation effectiveness*** provides further screening criteria to confirm whether enhanced aerobic bioremediation is likely to be effective. First, certain site-specific data on the nature/extent of contamination, potential risk to human health/the environment, subsurface geology and hydrogeology, and other relevant site

Exhibit XII-6
Initial Screening for Potential Effectiveness of Enhanced Aerobic Bioremediation



characteristics need to be evaluated. Next, the site-specific data must be compared to the criteria provided in the Exhibit to assess whether enhanced aerobic bioremediation is likely to be effective.

Step 3: ***An evaluation of the enhanced aerobic bioremediation system design*** in the corrective action plan allows a reviewer to determine whether basic design information has been defined, necessary design components have been specified, the construction process flow designs are consistent with standard practice, and adequate feasibility testing has been performed.

Step 4: ***An evaluation of the operation and monitoring plans*** allows a reviewer to determine whether baseline, start-up and long-term system operation and monitoring are of sufficient scope and frequency and whether remedial progress monitoring and contingency plans are appropriate.

Step 1 - Initial Screening of Enhanced Aerobic Bioremediation Effectiveness

This section reviews the initial screening tool to examine whether enhanced aerobic bioremediation is likely to be an effective approach to remediate the petroleum-impacted areas at a site. Before accepting enhanced aerobic bioremediation as the preferred remedial approach, determine whether the corrective action plan has taken into account key site-specific conditions. In addition, evaluate several "bright lines" that define the limits of enhanced aerobic bioremediation overall viability as a remedial technology. These bright lines will assist with evaluating the corrective action plan and determining whether enhanced aerobic bioremediation is appropriate as an appropriate solution. After establishing the overall viability of an enhanced aerobic bioremediation approach, look at basic site and petroleum contaminant information in order to further determine the expected effectiveness of enhanced aerobic bioremediation at the site.

Overall Viability

The following site conditions are considered to be the “bright lines” that define the general limits of enhanced aerobic bioremediation viability at a site. If review of the corrective action plan indicates that any of the following conditions exist, enhanced aerobic bioremediation is not likely to be a feasible or appropriate remedial solution for the site.

*Free mobile product is present and the corrective action plan does not include plans for its recovery*. Enhanced aerobic bioremediation will not effectively address free product that will serve as an on-going source of dissolved phase contamination. Biodegradation of the petroleum hydrocarbons occurs predominantly in the dissolved-phase because the compounds must be able to be transported across the microbial cell boundary along with water, nutrients, and metabolic waste products. Therefore, in the presence of free product, rates of hydrocarbon mass destruction using enhanced aerobic bioremediation will be limited by the rate at which the free product is dissolved into groundwater. The relatively low solubilities of petroleum hydrocarbon constituents will likely extend remediation for several years, and could allow further expansion of the contaminated area if free product is not removed. Additionally, some enhanced aerobic bioremediation technologies could actually spread the free product. For free product recovery approaches see *How to Effectively Recover Free Product At Leaking Underground Storage Tank Sites: A Guide for State Regulators,* US EPA 510-R-96-001, September 1996.

*Potentially excessive risks to human health or the environment have been identified and the corrective action plan does not include a supplemental mitigation plan.* While enhanced aerobic bioremediation can reduce petroleum hydrocarbon concentrations in the subsurface, site conditions may limit the level of such reductions and can significantly extend remedial timeframes. Close proximity of the petroleum contamination to basements, utilities, water supply wells, surface water bodies, or other potential receptorsthat could pose excessive risks should be mitigated using technologies that complement enhanced aerobic bioremediation (e.g., soil vapor extraction (SVE), hydraulic controls to protect water supply wells). Without the use of other remedial approaches, enhanced aerobic bioremediation may not be able to reduce concentrations of petroleum contaminants to sufficiently low concentrations to protect receptors in the predicted timeframes.

*The target contaminant zone includes unstratified dense clay.* For remedial success, enhanced aerobic bioremediation technologies must effectively introduce and distribute oxygen to indigenous microorganisms present in the treatment zone, allowing microbial populations to expand and metabolize the petroleum contaminants. With the relatively low permeabilities inherent to clay or clay-rich soils, oxygen and oxygen carrier media (e.g., air) cannot be easily introduced or distributed. Any distribution of oxygen that could be delivered to such soils (e.g., placement of oxygen releasing compounds in borings or excavations) would largely be controlled by molecular diffusion, a very slow and ineffective process. Treatment zone oxygen levels, therefore, would not be uniformly increased, and biodegradation of the petroleum hydrocarbons could not be effectively enhanced.

While these bright lines offer general guidance on the applicability of enhanced aerobic bioremediation technologies, there may be site-specific application-specific exceptions to the rule. It may be appropriate, for example, for enhanced aerobic bioremediation technologies to be used to address contamination on the periphery of contamination while a different technology is employed to treat the source zone.

Step 2 - Detailed Evaluation of Enhanced Aerobic Bioremediation Effectiveness

Potential Effectiveness of Enhanced Aerobic Bioremediation

Before performing a more detailed evaluation of enhanced aerobic bioremediation's potential saturated zone remedial effectiveness and future success at a site, it is useful to review several key indicators. Two factors influence the effectiveness of enhanced aerobic bioremediation at a site: saturated zone permeability, and biodegradability of the petroleum constituents.

- **Saturated soil permeability**. Soil permeability can strongly affect the rate at which oxygen is supplied and uniformly distributed to the hydrocarbon-degrading bacteria in the subsurface. Enhanced aerobic bioremediation of groundwater contaminants in fine-grained soils, or in clays and silts with low permeabilities, is likely to be less effective than in coarse-grained soils (e.g., sand and gravels) because it is more difficult to effectively deliver oxygen in low-permeability materials. In coarse-grained soils, oxygen can be more easily delivered to bacteria, and beneficial populations of hydrocarbon-degrading bacteria may come into contact with more of the petroleum, which enhances biodegradation.

- **Biodegradability**. Biodegradability is a measure of a contaminant's propensity to be metabolized by hydrocarbon-degrading microorganisms. Petroleum products are generally biodegradable, as long as indigenous microorganisms have an adequate supply of oxygen and nutrients. However, the rate and degree to which petroleum products can be degraded by the microorganisms present in the subsurface is largely determined by the relative biodegradability of the petroleum products. For example, heavy petroleum products (e.g., lubricating oils, fuel oils) generally contain a higher proportion of less soluble, higher molecular weight petroleum constituents that are biodegraded at a slower rate than more soluble, lighter fraction petroleum compounds (e.g., gasoline). As a general rule, these characteristics of petroleum compounds can limit biodegradation rates.

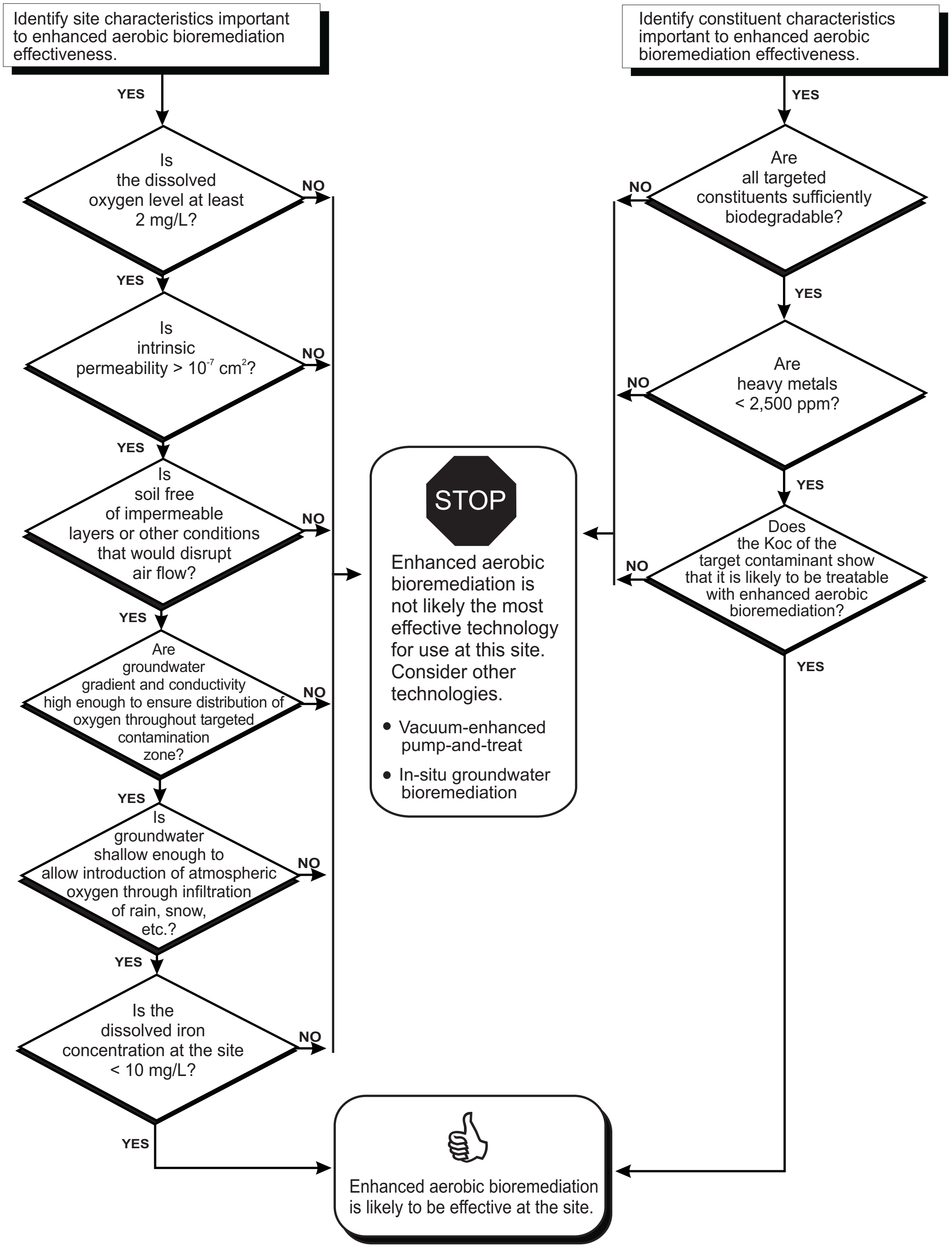
> Less soluble compounds are generally less available in the aqueous phase for microorganisms to metabolize. Larger petroleum molecules can slow or preclude the transport of some of these molecules into microbial cells for degradation, and larger or longer chain length structural properties may hinder the ability of the micro-organisms' enzyme systems to effectively attack the compounds. Therefore, even under identical site conditions, bioremediation of a lubricating oil spill will generally proceed more slowly than at a gasoline release. However, cleanup goals are frequently tied to specific petroleum compounds rather than the range of organic constituents that may comprise a petroleum product. Therefore, when considering enhanced aerobic bioremediation, the biodegradability of specific petroleum compounds common to the petroleum product and cleanup goals are of greatest relevance. Even though bioremediation of lubricating oil contamination may occur relatively slowly, cleanup of a lubricating oil spill site via bioremediation may be achieved more quickly than bioremediation of a gasoline spill site because fewer compounds in lubricating oil dissolve in groundwater, reducing the number of target species to clean up.

Some chemical species present in gasoline, such as methyl tertiary-butyl ether (MTBE), are more recalcitrant to bioremediation than are some of the heaviest and most chemically complex petroleum compounds. The detailed enhanced aerobic bioremediation effectiveness evaluation section of this chapter consider the biodegradability of specific petroleum hydrocarbon constituents, such as the benzene, toluene, ethylbenzene, and xylene (BTEX) compounds, as well as that of fuel oxygenates, such as MTBE.

The following section provides information needed to make a more thorough evaluation of enhanced aerobic bioremediation effectiveness and help to identify areas that may require special design considerations. Exhibit XII-7 provides a stepwise process that reviewers should use to further evaluate whether enhanced aerobic bioremediation is an appropriate technology for a contaminated UST site. To use this tool, determine the type of soil present and the type of petroleum product released at the site.

To help with this more detailed evaluation, this section covers a number of important site-specific characteristics influencing the potential effectiveness of enhanced aerobic bioremediation that were not considered or fully explored in the initial screening of the remedial approach. Additionally, this section provides a more detailed discussion of key contaminant characteristics that influence the potential effectiveness of enhanced aerobic bioremediation. Key site and contaminant factors that should be explored in the detailed evaluation of enhanced aerobic bioremediation are listed in Exhibit XII-8. The remainder of this section details each of the parameters described in Exhibit XII-8. After reviewing and comparing the information provided in this section with the corresponding

Exhibit XII-7
Detailed Screening for Potential Effectiveness of Enhanced Aerobic Bioremediation



information in the corrective action plan, it should be possible to evaluate whether enhanced aerobic bioremediation is likely to be effective at the site.

Exhibit XII-8
Key Parameters Used to Evaluate Enhanced Aerobic Bioremediation Applicability

Site Characteristics	Constituent Characteristics
Oxygen Demand Factors # Five-Day Biological Oxygen Demand (BOD$_5$) # Contaminant theoretical oxygen demand # Naturally occurring organic material (humic substances) – Microbial population density/activity – Nutrient concentrations – Temperature – pH	*Chemical Class and Susceptibility to Bioremediation* *Contaminant Phase Distribution* *Concentration and Toxicity*
Advective and Dispersive Transport Factors # Intrinsic permeability # Soil structure and stratification # Hydraulic gradient # Depth to groundwater # Dissolved iron content	*Bioavailability Characteristics* # Solubility # Organic carbon partition coefficient (K$_{oc}$)/sorption potential

Site Characteristics Affecting Enhanced Aerobic Bioremediation

The effectiveness of enhanced aerobic bioremediation depends largely on the ability to deliver oxygen to naturally occurring hydrocarbon-degrading microorganisms in the target treatment area. Oxygen can be introduced and removed from a contaminated groundwater zone in many different ways. Dissolved oxygen may enter the contaminated zone from any of the following sources:

Flow of groundwater into the contaminated zone from background (upgradient) areas
Precipitation infiltration
Other enhanced aerobic bioremediation sources

Losses of oxygen from the contaminated zone may occur through:

- Biodegradation of organic contaminants
- Oxidation of naturally occurring organic and inorganic material in the soil
- Volatilization of dissolved oxygen
- Flow of groundwater containing depleted levels of dissolved oxygen leaving the contaminated zone

The success of enhanced aerobic bioremediation, therefore, hinges on the balance between oxygen sources, oxygen uptake, and the degree to which the transport of dissolved oxygen in groundwater is limited. To support aerobic biodegradation of petroleum contaminants, the most favorable dissolved oxygen (DO) level is 2 mg/L or higher. Anaerobic biodegradation processes in the anaerobic shadow become limited once dissolved oxygen levels approach or fall below 2 mg/L. Site characteristics affecting the delivery and distribution of oxygen in the subsurface and the effectiveness of enhanced aerobic bioremediation technology are discussed in the following sections.

Oxygen Demand Factors. Groundwater in petroleum spill source area and downgradient of the spill area is usually depleted of oxygen. This zone of oxygen-depleted groundwater, commonly referred to as the anaerobic shadow, results from the use of oxygen by naturally occurring microorganisms during aerobic metabolism of the spilled petroleum organic compounds. The oxygen is used in the microbiologically mediated oxidation of the petroleum contaminants. Aerobic biodegradation processes in the anaerobic shadow become limited once dissolved oxygen levels approach or fall below 2 mg/L. Enhanced aerobic bioremediation technologies can boost oxygen levels in the source area and in the anaerobic shadow to assist naturally occurring aerobic biodegradation processes but there are other oxygen demands that need to be considered before attempting to oxygenate the anaerobic shadow.

Each enhanced aerobic bioremediation technology has a particular way of delivering oxygen to the saturated zone. Once delivered to the saturated zone, dissolved oxygen can be further distributed in the treatment zone by groundwater advection and dispersion. However, from the point where it is introduced into the aquifer, dissolved oxygen concentration decreases along the groundwater flow path not only through mixing with the oxygen-depleted groundwater, but also because of biologically mediated and abiotic oxidation processes. The rate and degree to which oxygen concentrations decrease along the groundwater flow path and the degree to which the anaerobic shadow may be oxygenated depends, in part, on the degree to which oxygen is lost to microbiological and abiotic consumption in the saturated zone.

Demand for oxygen in the subsurface environment may stem from organic or inorganic sources. Microbial biodegradation of released petroleum hydrocarbons or naturally occurring organics (e.g., humic substances) as a carbon source by aerobic microorganisms will generate demand for oxygen.

Oxygen Demand From Biodegradation of Organic Compounds. Oxygen levels are generally depleted in the subsurface, but are particularly depleted at petroleum UST spill sites. This oxygen shortage results from the relative isolation of the subsurface from the oxygen-replenishing atmosphere, as well as the oxygen demands of naturally occurring organic and inorganic compounds and petroleum hydrocarbon releases. Because of these oxygen-depleted conditions, the most basic requirement for enhanced aerobic bioremediation is to deliver sufficient levels of oxygen to maintain an aerobic subsurface environment.

Exhibit XII-9 outlines the stoichiometric reactions for the complete oxidation or biodegradation of some common components of gasoline and other petroleum products. In theory, oxygen levels of at least 3 to 3.5 times the amount of subsurface petroleum mass that needs to be removed to meet cleanup goals must be delivered to the groundwater and distributed over the planned remedial period. Given typical oxygen solubility limits and the mass of contaminants that are often found at leaking underground storage tanks sites, delivering the required amount of oxygen can be a significant challenge. In practice, to convert one pound of hydrocarbon material into carbon dioxide and water requires between 3 and 5 pounds of available oxygen. This is valuable for evaluating the potential effectiveness of enhanced aerobic bioremediation.

Exhibit XII-9
Organic Compound Oxidation Stoichiometry

Petroleum Hydrocarbon	Oxidation Reaction	Oxygen Requirement (gram O_2 per gram Contaminant)
Benzene	$C_6H_6 + 7.5\ O_2 \rightarrow 6CO_2 + 3H_2O$	3.1
Toluene	$C_6H_5CH_3 + 9\ O_2 \rightarrow 7CO_2 + 4H_2O$	3.1
Ethylbenzene	$C_2H_5C_6H_5 + 10.5\ O_2 \rightarrow 8CO_2 + 5H_2O$	3.2
Xylenes	$C_6H_4(CH_3)_2 + 10.5\ O_2 \rightarrow 8CO_2 + 5H_2O$	3.2
Cumene	$C_6H_5C_3H_7 + 12O_2 \rightarrow 9O_2 + 6H_2O$	3.2
Naphthalene	$C_{10}H_8 + 12O_2 \rightarrow 10CO_2 + 4H_2O$	3.0
Fluorene	$C_{13}H_{10} + 15.5O_2 \rightarrow 13CO_2 + 5H_2O$	3.0
Phenanthrene	$C_{14}H_{10} + 16.5O_2 \rightarrow 14CO_2 + 5H_2O$	3.0
Hexane	$C_6H_{14} + 9.5\ O_2 \rightarrow 6CO_2 + 7H_2O$	3.5

Because the solubility of O_2 by natural oxygen replenishment is limited and relatively low (9 mg/L at 25°C), only a small amount of organic or inorganic matter in the subsurface can consume all the naturally present dissolved O_2 in groundwater. For example, using the above stochiometric equation for the complete oxidation of benzene, oxidation of 2.9 mg/L of benzene would theoretically consume about 9 mg/L of O_2, leaving no residual oxygen in the water. It can be readily understood how external sources of oxygen enhanced aerobic bioremediation technologies can help aerobic bacteria by providing a source of energy so they may consume the petroleum as a source of carbon.

Microbial Population. Oxygen demand is also a function of the vitality of the microbial population. The larger and more active the population of aerobic microorganisms, the larger the biological oxygen demand. However, subsurface conditions may not be conducive to producing large numbers of microbial populations. Exhibit XII-10 shows the likely effectiveness of enhanced aerobic bioremediation as a function of the presence of heterotrophic bacteria in the subsurface.

Exhibit XII-10
Relationship Between Heterotrophic Bacterial Counts And Likely Enhanced Aerobic Bioremediation Effectiveness

Background Heterotrophic Bacteria Levels	Enhanced Aerobic Bioremediation Effectiveness
>1,000 CFU/gram dry soil	Generally effective
<1,000 CFU/gram dry soil	May be effective; further evaluation needed to determine if toxic conditions are present

Nutrients. The activity of the microbial population and the corresponding biological oxygen demand also depend on the availability of inorganic nutrients such as nitrogen and phosphate to support cell growth and sustain biodegradation processes. Nutrients may be initially available in sufficient quantities in the aquifer, but with time, they may need to be supplemented with additional nutrient loading to maintain adequate bacterial populations. Excessive amounts of certain nutrients (e.g., phosphate or sulfate) can repress bio-metabolism. The carbon:nitrogen:phosphorus ratios necessary to enhance biodegradation fall in the range of 100:10:1 to 100:1:0.5, depending on the constituents and bacteria involved in the biodegradation process.

However, to avoid over-application of nitrogen and phosphorus, which can unnecessarily incur added costs, plug wells, and even contaminate ground water with nitrate, it is important to understand how much carbon can be metabolized based on oxygen-limiting conditions. Nitrogen and phosphorus should be added to reach the proportions identified in the previous paragraph, based on the amount of carbon that can be metabolized at any given time compared to the total average concentration of carbon (i.e., petroleum contamination) in the subsurface. For example, if during full-scale operation a net 0.6 pound per hour of pure oxygen is introduced to the treatment area and is assumed to be completely consumed by aerobic microbial activity, approximately 0.17 pound per hour (4 pounds per day) of hydrocarbon is theoretically microbiologically oxidized (using a 3.5:1 oxygen:hydrocarbon stoichiometric ratio). Then, using the 100:10:1 to 100:1:0.5 C:N:P theoretically optimal ratio range for this example, between 0.4 and 0.04 pounds per day of nitrogen and 0.04 to 0.02 pounds per day of phosphorus may need to be added to the treatment area to keep up with the estimated carbon metabolism rate.

Alternatively, it would be reasonable for a practitioner to suggest monitoring oxygen demand during full-scale system operation before considering adding any nitrogen or phosphorus. If oxygen demand were to fall below about 10 mg/L in the petroleum contaminated area, the subsurface could be tested for nitrogen or phosphorus to determine whether insufficient concentrations of these micronutrients is limiting microbial activity. Only after this determination is made should nitrogen or phosphorus be added. Generally, nitrogen should not limit aerobic degradation processes unless concentrations fall significantly below 1 mg/L. This alternative may be particularly attractive at sites located near areas where aquifers already have nitrogen problems because it may be difficult to secure permits for the injection of these micronutrients. If nitrogen addition is necessary, slow-release sources should be used. Nitrogen addition can lower pH, depending on the amount and type of nitrogen added.

pH. Although the optimum pH for bacterial growth is approximately 7, enhanced aerobic bioremediation can be effective over a pH range of 5 to 9 pH units. Adjustment of pH conditions outside this range is generally not considered to be viable because it is difficult to overcome the natural soil buffering capacity, and because of the potential for rapid changes in pH to adversely affect bacterial populations. Oxygen releasing compounds may raise the pH even higher than the 5-9 range, which can be fatal to microbes.

Temperature. Oxygen uptake and bacterial growth rate are directly affected by temperature. From 10°C to 45°C, the rate of microbial activity typically doubles for every 10°C rise in temperature. Below 5°C, microbial activity becomes insignificant. In most areas of the United States, the average groundwater temperature is about 13°C. Groundwater temperatures may be somewhat lower or higher in the extreme northern and southern states. While individual microorganism growth rates decrease with temperature, a higher steady state biomass of active organisms (each one working more slowly, but more of them working) can result from lower temperatures. Because of this and the increased solubility of oxygen at lower temperatures, biodegradation can sometimes be as fast or faster at lower temperatures than at more moderate temperatures.

Inorganic Oxygen Demand. Oxygen demand arises from a depletion of subsurface oxygen from biological or inorganic processes coupled with poor oxygen replenishment. In contrast to surface water bodies, groundwater systems are typically isolated from the atmosphere, limiting the opportunity for natural oxygen to be replenished. This atmospheric isolation allows dissolved oxygen levels to become depleted and subsurface conditions to become geochemically reduced. Introducing and distributing oxygen under these reduced conditions are challenging for the application of enhanced aerobic bioremediation, because introduced oxygen may react with and become lost to organic or inorganic chemical constituents that would otherwise be relatively inconsequential to the environmental cleanup.

Exhibit XII-11 presents a sample of some common inorganic processes that consume oxygen in groundwater.[3] Corrective action plan data should be reviewed to identify what is already known about aquifer conditions in the area around the site to determine whether signficant reduced inorganic species exist in the subsurface that could remove oxygen from groundwater. If so, these species can limit the ability of biodegrading bacteria to effectively implement enhanced aerobic bioremediation. In such cases, soil core samples may need to be collected and analyzed for reduced iron, sulfide or other inorganic constituents. These samples can help to determine the potential loss of oxygen to the aquifer and to verify that enhanced aerobic bioremediation will be able to effectively deliver sufficient oxygen to overcome these limiting factors. This assessment cannot be made from analyses of groundwater samples, because the reduced inorganic complexes are primarily precipitated in the aquifer material.

Exhibit XII-11
Inorganic Oxidation Processes That Consume Dissolved Oxygen In Groundwater

Process	Reaction
Sulfide Oxidation	$O_2 + ½HS^- \rightarrow ½SO^{2-} + ½H^+$
Iron Oxidation	$¼O_2 + Fe^{+2} + H^+ \rightarrow Fe^{+3} + ½H_2O$
Nitrification	$O_2 + ½NH^{4+} \rightarrow ½NO^{3-} + H^+ + ½H_2O$
Manganese Oxidation	$O_2 + 2Mn^{2+} + 2H_2O \rightarrow 2MnO_2$ (s) $+4H^+$
Iron Sulfide Oxidation	$15/4O_2 + FeS_2$ (s) $+ 7/2H_2O \rightarrow Fe(OH)_3$ (s) $+2SO_4^{2-} + 4H^+$

Many inorganic oxygen-consuming reactions produce solid precipitates that can accumulate in soil pore spaces. As discussed below, these precipitates can restrict soil permeabilities and thus further affect the ability of enhanced aerobic bioremediation technologies to deliver and distribute oxygen to hydrocarbon-degrading microorganisms.

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[3] From Freeze R.A. and John A. Cherry, 1979. Groundwater. Prentice Hall.

Advective and Dispersive Transport Factors. The site conditions affecting advection and dispersion of dissolved oxygen are outlined below. These conditions are:

- Intrinsic permeability
- Soil structure and stratification
- Hydraulic gradient
- Depth to groundwater
- Iron and other reduced inorganic compounds dissolved in groundwater

Each of these factors is described in more detail below.

Intrinsic Permeability. Intrinsic permeability is a measure of the ability of soil to transmit fluids. Intrinsic permeability is the single most important soil characteristic in determining the effectiveness of enhanced aerobic bioremediation, because intrinsic permeability controls how well oxygen can be delivered and dispersed to subsurface microorganisms. Hydraulic conductivity is a measure of the resistance of aquifer material to groundwater flow. This unit of measure is particularly relevant to understanding the ability to move oxygen dissolved in groundwater through the saturated treatment zone. Hydraulic conductivity is related to intrinsic permeability by the following equation.

$$K = \frac{k g}{m}$$

where:
K = hydraulic conductivity (L/T)
k = intrinsic permeability (L^2)
g = weight density of water (F/L^3)
m = dynamic viscosity of water ($F \cdot T/L^2$)
L = mean grain diameter
T = transmissivity
F = fluid density

Intrinsic permeability often decreases near injection wells or infiltration galleries. This also commonly results from precipitation of carbonates, or precipitates of other minerals derived from fertilizer solutions. In general, oxygen is more easily distributed in soils with higher soil permeabilities (e.g., coarse-grained soils such as sands) than in soils with lower permeabilities (e.g., fine-grained clayey or silty soils).

Calculation of intrinsic permeability can be derived from hydraulic conductivity measurements taken from on-site pump testing. Pump test or slug test-derived permeability ranges are typically representative of average hydraulic permeability conditions for heterogeneous conditions. Alternatively, intrinsic permeability can be estimated from soil boring logs.

Permeabilities derived from pump or slug test analyses or estimated from boring logs are only approximations of actual subsurface conditions and should be regarded as such in the evaluation of enhanced aerobic bioremediation potential effectiveness.

Intrinsic permeability can vary over 13 orders of magnitude (from 10^{-16} to 10^{-3} cm^2) for the wide range of earth materials. Exhibit XII-12 provides general guidelines on the range of intrinsic permeability values over which enhanced aerobic bioremediation is likely to be effective.

The intrinsic permeability of a soil is likely to decrease as enhanced aerobic bioremediation progresses. If the soil intrinsic permeability indicates borderline potential effectiveness (e.g., $10^{-6} \leq k \leq 10^{-7}$), the geochemistry should be further evaluated.

Exhibit XII-12

Intrinsic Permeability And Enhanced Aerobic Bioremediation Effectiveness

Hydraulic Conductivity (K) (in ft/s)	Intrinsic Permeability (k) (in ft^2)	Enhanced Aerobic Bioremediation Effectiveness
$K > 10^{-6}$	$k > 10^{-12}$	Effective to generally effective
$10^{-6} \leq K \leq 10^{-7}$	$10^{-12} \leq k \leq 10^{-13}$	Possibly effective; needs further evaluation
$K < 10^{-7}$	$k < 10^{-13}$	Marginally effective to ineffective

Soil Structure and Stratification. Often, soils in a target treatment area are not uniformly permeable (heterogeneous), but rather have large-scale or small-scale variations in permeability. Soil heterogeneity plays a very important role in enhanced aerobic bioremediation technologies because oxygen introduced to the subsurface is distributed preferentially along higher permeability layers in the saturated soil. For example, in a heterogeneous soil comprised of sand, silt and clay layers, oxygen may be effectively distributed through the sand layer to successfully reduce petroleum hydrocarbons there, but will be ineffectively delivered and distributed to the silt and clay layers. The relatively slow diffusion transport mechanism will become as important or more important than advection and dispersion in the distribution of oxygen to microorganisms in the silt and clay layers. If the silt and clay layers are thick relative to the sand horizon and contain significant petroleum hydrocarbon mass, enhanced aerobic bioremediation technologies may not be efficient or effective. In this case, the dissolved petroleum hydrocarbon mass will appear to shrink as the most permeable zone (i.e., the sand) will have undergone significant enhanced aerobic bioremediation treatment.

However, the petroleum mass in the silt and clay horizons will likely not biodegrade, and will also likely diffuse into the sand zone, causing a rebound in dissolved hydrocarbon concentrations at the site.

Unless site soils are homogeneous, average soil intrinsic permeability may not adequately determine the viability of enhanced aerobic bioremediation approaches because discrete low permeability soil horizons may exist, and these horizons might contain a large fraction of the subsurface petroleum mass.   In most cases, it is prudent to evaluate petroleum mass distribution across all soil types to determine whether enhanced aerobic bioremediation is likely to be effective and will achieve cleanup objectives. If select soil horizons containing hydrocarbon mass are not expected to be effectively treated using enhanced aerobic bioremediation , enhanced aerobic bioremediation may not be viable for the site. For example, if 50% of the contaminant mass is contained and isolated in low permeability soil horizons and the site cleanup goals is a 95% reduction in petroleum contaminant concentrations, then it is reasonable to conclude that the goal cannot be achieved using enhanced aerobic bioremediation. However, in such circumstances, combining enhanced aerobic bioremediation with other technologies that enhance the permeability of low permeability horizons in the contaminated zone (e.g., soil fracturing) could be considered. Soil fracturing could allow dissolved oxygen and other microbial nutrients to be effectively delivered through the engineered fractures in low permeability soil. However caution should be observed when considering this option because the same fractures produced to enhance permeability for nutrient delivery could also be a potential preferential flow path for contaminant plume migration.

Hydraulic Gradient. Enhanced aerobic bioremediation technologies ultimately rely on groundwater advection and dispersion (i.e., flow) to distribute dissolved oxygen to the subsurface. Distribution of introduced dissolved oxygen is most effective under hydrogeologic conditions conducive to higher groundwater flow rates. These conditions exist when the combined values of hydraulic gradient and hydraulic conductivity are relatively high.

Note that state regulations may either require permits for nutrient injection or prohibit them entirely. Depending on the specific enhanced aerobic bioremediation technology and the state in which the site is located, permits that may be required include underground injection, treated groundwater discharge (to sanitary or storm sewer, or air (soil vapor) discharge. Several federal, state and local programs exist that either directly manage or regulate Class V aquifer remediation wells, and many of these require permits for underground injection of oxygen or bionutrients.

As the hydraulic gradient increases, the groundwater velocity increases proportionately. This same relationship exists between groundwater velocity and soil permeability. Groundwater velocity is inversely proportional to soil porosity. As porosity increases, groundwater velocity decreases. For purposes of evaluating the feasibility of using an enhanced aerobic bioremediation technology, keep in mind that the principal direction of groundwater flow and oxygen transport is along the line of maximum hydraulic gradient.

To maximize the distribution of dissolved oxygen through and biodegradation rates in the contaminated zone, enhanced aerobic bioremediation technologies often introduce dissolved oxygen at levels that exceed the solubility limit of oxygen in groundwater under atmospheric conditions. However, when the oxygen is not rapidly dissipated or used (e.g., as an electron acceptor during microbial respiration), the oxygen can partition out of the dissolved-phase and be lost to the unsaturated zone as a gas.

Depth to Groundwater. The depth to groundwater at a site can also affect the availability and transport of dissolved oxygen to the subsurface. Infiltrating precipitation, such as rainfall or snow, is a source of dissolved oxygen to the saturated zone. When groundwater is relatively deep or confined, less precipitation infiltrates, minimizing the amount of atmospheric dissolved oxygen that reaches the groundwater. Also, pavement prevents infiltration of rainfall or snowmelt At sites where the water table is close to the surface, more mixing of groundwater with air-saturated precipitation occurs, resulting in more opportunity for groundwater to be oxygenated. When this occurs, dissolved oxygen levels in groundwater can even approach those found in streams and other surface water bodies.

Iron and Other Reduced Inorganic Compounds Dissolved in Groundwater. In addition to being a significant oxygen sink, the effective intrinsic permeability of the saturated zone can be significantly reduced if the enhanced aerobic bioremediation treatment zone contains naturally elevated levels of reduced iron (e.g., ferrous iron, or Fe^{+2}) or other mineral species. The net impact of elevated levels of reduced species can therefore be a loss of delivered oxygen and a decreased ability to distribute any excess oxygen to the aerobic microorganisms involved with the degradation of the petroleum hydrocarbons. Precipitation of oxidized inorganic complexes and biological mass can foul monitoring and injection well screens and potentially aquifer pore space where oxygen is delivered to the subsurface.

Exhibit XII-13 can be used as a guide to help determine whether the corrective action plan has considered site levels of dissolved iron and if dissolved iron levels at the site could have an adverse effect on the enhanced aerobic bioremediation approach.

In some situations, hydraulic gradients can be enhanced to help increase groundwater flow and oxygen delivery rates and flush dissolved oxygen through the contaminated zone. One common approach is to create an artificial gradient by removing groundwater downgradient of the source area, treating it, and re-introducing it in the upgradient source area. For example, hydrogen peroxide enhanced aerobic bioremediation applications often require extracting

Exhibit XII-13
Relationship Between Dissolved Iron And Enhanced Aerobic Bioremediation Effectiveness

Dissolved Iron Concentration (mg/L)	Potential Effectiveness of Enhanced Aerobic Bioremediation
$Fe^{+2} < 10$	Enhanced aerobic bioremediation will likely be effective.
$10 \geq Fe^{+2} \geq 20$	Enhanced aerobic bioremediation injection wells and delivery systems will require periodic testing and may need periodic replacement.
$Fe^{+2} > 2$	Enhanced aerobic bioremediation may not be cost effective due to loss of dissolved oxygen to the formation and equipment maintenance problems associated with inorganic precipitation. This would especially be the case where groundwater is extracted, treated, amended with oxygen (e.g., hydrogen peroxide) and reinjected.

contaminated groundwater from the downgradient portion of the dissolved hydrocarbon plume, treating the extracted groundwater for hydrocarbons, and re-injecting the treated groundwater amended with hydrogen peroxide into one or more upgradient locations.

This lowers the groundwater level in the downgradient extraction locations and raises it in upgradient injection locations, which provides an artificially increased gradient. This, in turn, increases the rate of groundwater and oxygen flow across the contaminated zone.

Even with preferential hydrogeologic conditions, distributing dissolved oxygen throughout the subsurface is difficult because of the inherent limits of groundwater flow and the number of oxygen “sinks,” or uptakes, that can exist, particularly in areas contaminated with petroleum hydrocarbons. These limitations frequently require that the corrective action plan call for placement of a large number of oxygen delivery points in the treatment area to decrease enhanced aerobic bioremediation technology’s reliance on groundwater flow as the principal source of distributed oxygen.

In addition to being a parameter considered in evaluating the potential effectiveness of enhanced aerobic bioremediation, hydraulic gradient is an engineering design issue. If the gradient is not steep enough to provide adequate flow and oxygen transport through the contaminated zone, then certain engineering provisions (e.g., spacing application points more closely, creating artificial hydraulic gradients) can be added to the design to enhance oxygen distribution. However, economic considerations limit the extent to which design changes can be made in an enhanced aerobic bioremediation delivery system to ensure adequate oxygen distribution.

Constituent Characteristic Affecting Enhanced Aerobic Bioremediation

It is important to evaluate the potential impacts of site contaminants on the performance of the proposed enhanced aerobic bioremediation approach. In particular, it is important to review how the chemical structure, chemical properties, concentrations and toxicities of the petroleum contaminants can influence remedial performance.

Chemical Class and Susceptibility to Bioremediation. Petroleum products are complex mixtures of hundreds or even thousands of hydrocarbon chemical constituents, other chemical constituents and additives. Each of these constituents has a different atomic structure that determines, in part, its relative biodegradability. Although nearly all constituents in petroleum products found at leaking underground storage tank sites are biodegradable to some extent, constituents with more complex molecular structures are generally less readily biodegraded than those with simpler structures. On the other hand, most low-molecular weight (nine carbon atoms or less) aliphatic and monoaromatic constituents are more easily biodegraded than higher molecular weight aliphatic or polyaromatic organic constituents.

Exhibit XII-14 lists the relative biodegradability of various petroleum products and constituents. The exhibit shows that hydrocarbon molecules containing a higher number of carbon atoms (e.g., lubricants with 26- to 38-carbon chains) degrade more slowly, and perhaps less completely, than those with shorter carbon chains (e.g., gasoline). However, cleanup goals are frequently tied to a small subset of chemical compound components of the various petroleum products in Exhibit XII-9 rather than a total petroleum hydrocarbon concentration. Often chemical compounds in petroleum products identified in Exhibit XII-14 as being less readily biodegradable are not present at contaminated sites at levels significantly above cleanup standards because of the low solubility characteristic that these compounds can have. Consequently, cleanup standards for contaminants in less readily biodegradable petroleum formulations may be reached through enhanced aerobic bioremediation more quickly than those for more soluble compounds in more biodegradable formulations.

Certain petroleum constituents are more recalcitrant than most other constituents. For example, MTBE, a gasoline additive, is frequently found at leaking UST sites because of its environmental persistence and its apparent resistance to bioremediation. Some researchers have estimated that the half-life of MTBE in the environment is at least two years, whereas the typical half-life for BTEX compounds in the environment is approximately two to three months.

Exhibit XII-14
Composition And Relative Biodegradability Of Petroleum Products

Product	Major Components	Relative Product Biodegradability
Natural Gas	Normal and branched-chain alkanes. One to five carbons in length. *Examples: ethane, propane.*	**Higher**
Gasoline	Normal and branched hydrocarbons between 6 and 10 carbons in length. *Examples: n-butane, n-pentane, n-octane, isopentane, methylpentanes, benzene, toluene, xylenes, ethylbenzene*.	
Kerosene, Diesel	Primarily 11 to 12 carbon hydrocarbons, although the range of carbons extends well above and below this range. Generally contains low to non-detectable levels of benzene and polyaromatic hydrocarbons. Jet fuel oils have a similar composition. *Examples: n-nonane, n-decane, n-dodecane, naphthalene, n-propylbenzene*.	
Light Gas Oils (e.g., No 2 Fuel Oil)	Twelve to 18 carbon hydrocarbons. Lower percentage of normal alkanes than kerosene. These products include diesel and furnace fuel oils (e.g., No. 2 fuel oil). *Examples: fluorene, naphthalene, phenanthrene, isopropylbenzene*.	
Heavy Gas Oils and Light Lubricating Oils	Hydrocarbons between 18 and 25 carbons long.	
Lubricants	Hydrocarbons between 26 and 38 carbons long.	
Asphalts	Heavy polycyclic compounds.	**Lower**

Therefore, one should carefully consider the biodegradability of the target contaminants when forecasting the potential effectiveness and usefulness of an enhanced aerobic bioremediation technology. The enhanced aerobic bioremediation design and implementation should focus on the most recalcitrant compounds within the released petroleum product, unless another remedial technology is being proposed to address those compounds.

It is not necessarily the most recalcitrant or most difficult compound to bioremediate that determines the duration of a remediation project. For example, the baseline concentration of the most recalcitrant site compound may be much closer to its respective cleanup goal or an acceptable risk-based concentration than a more readily biodegradable petroleum constituent at a baseline level much greater than its cleanup goal. In this case, the more biodegradable constituent may initially be the focus of the enhanced aerobic bioremediation design and cleanup. As remediation progresses, the mix of petroleum products remaining should periodically be compared to the site's proposed cleanup level to determine whether the remedial approach needs to be enhanced to address the remaining target compounds.

Researchers have estimated and published biodegradation rate constants for various petroleum hydrocarbons. These rate constants can indicate the relative biodegradability of petroleum hydrocarbon constituents under field conditions. However, actual degradation rates for target contaminants may depend on constituent-, site-, and enhanced aerobic bioremediation implementation-specific conditions. For example, the mixture and concentrations of the different petroleum constituents in the site soil and groundwater may play an important role in determining relative degradation rates. The amount of natural organic matter in the soil and the degree to which the petroleum constituents attach themselves to it will affect the relative rates of biodegradation. These issues, especially as they relate to contaminant characteristics that affect aerobic bioremediation, are discussed below.

Contaminant Phase Distribution. Spilled petroleum products may be partitioned into one or more phases and zones in the subsurface including:

- Unsaturated soils (sorbed phase)
- Saturated soil (sorbed phase)
- Dissolved in groundwater (aqueous phase)
- Unsaturated soil pore space (vapor phase)
- Free mobile product (liquid phase)
- Free residual product smeared onto soil above and below the water table

Understanding how the petroleum contaminant mass is distributed in the subsurface can be important to both evaluating the applicability of enhanced aerobic bioremediation and identifying a particular enhanced aerobic bioremediation technology that will be effective. Depending on site-specific cleanup goals and contaminant levels, a disproportionate amount of contaminant mass in one medium or another could preclude the use of enhanced aerobic bioremediation technologies. For example, if a relatively large portion of the mass of a site target compound (e.g., benzene) is held in residual free product that is vertically smeared above and below the water table, enhanced aerobic bioremediation may not be able to achieve the site cleanup goals within a reasonable period of time. However, in such a case, enhanced aerobic bioremediation could still potentially be used at the fringes of the contaminated area while a more aggressive technology is employed in the residual-free product zone.

Information on the distribution of target compounds in the subsurface can also be used to help identify the most appropriate enhanced aerobic bioremediation technology for a site. Depending on where most of the target contaminant mass is located, one or more of the enhanced aerobic bioremediation technologies may be viable. For example, a disproportionate amount of target contaminant mass in the unsaturated soil would logically lead to the selection of an unsaturated zone enhanced aerobic bioremediation approach (e.g., bioventing). On the other hand, if a disproportionate amount of target contaminant mass is in the saturated zone, one of the enhanced aerobic bioremediation technologies that introduces high concentrations of dissolved oxygen to the subsurface may be a reasonable approach.

Concentration and Toxicity. High concentrations of petroleum organics or heavy metals in site soils and groundwater have traditionally been thought to be potentially toxic to, or inhibit growth and reproduction of, biodegrading bacteria. Soil containing petroleum hydrocarbons in amounts greater than 50,000 ppm, or heavy metals in excess of 2,500 ppm, was thought to be inhibitory and/or toxic to many aerobic bacteria. However, it is becoming increasingly evident that many microorganisms are able to tolerate and adapt to petroleum concentrations well above 50,000 ppm. Some researchers have even reported being able to isolate living bacteria directly from gasoline product.

While it appears that bacteria may be more adaptable than initially believed, to the extent that these higher levels of petroleum hydrocarbons represent a large mass of contamination in unsaturated or saturated soil in contact with groundwater, the adapted populations of bacteria may not be able to address the contaminant mass in a reasonable timeframe. When considering the feasibility of enhanced aerobic bioremediation, it is important to evaluate the mass of the target contaminants of concern relative to potential biodegradation rates and the cleanup timeframe objective.

It is possible that the effects of elevated contaminant levels can include partial biodegradation of only a fraction of the hydrocarbons at reduced rates, or reduced bacterial reproduction rates or metabolism, resulting in minimal or no appreciable soil treatment. The guidance threshold values summarized in Exhibit XII-15 can be compared to average site concentrations provided in the corrective action plan as another way of forecasting the potential effectiveness of enhanced aerobic bioremediation. Again, it is important to recognize that the values shown in Exhibit XII-15 are guidance values only.

As outlined in Exhibit XII-15, the threshold petroleum concentrations above which biodegradation is inhibited could also indicate the presence of free or residual product in the subsurface. In the initial effectiveness screening of enhanced aerobic bioremediation (Step 1), one of the feasibility bright lines discussed was the absence of free mobile product. If threshold soil petroleum levels exist, then free or residual petroleum product most likely exists in the soil, and enhanced aerobic bioremediation will not be effective without first removing the product through other remedial measures.

Exhibit XII-15
Constituent Concentration and Enhanced Aerobic Bioremediation Effectiveness

Contaminant Levels (ppm)	Enhanced Aerobic Bioremediation Effectiveness
Petroleum constituents $\leq$ 50,000 Heavy metals $\leq$ 2,500	Possibly effective
Petroleum constituents > 50,000 or Heavy metals > 2,500	Not likely to be effective either due to toxic or inhibitory conditions to bacteria, or difficulty in reaching cleanup goal within reasonable period of time

Bioavailability Characteristics. The extent to which and the rate at which a particular petroleum hydrocarbon compound can be biodegraded by microorganisms depends not only on the compound's inherent biodegradability, but also on the availability of the compound to hydrocarbon-degrading bacteria ("bioavailability"). Several contaminant properties contribute to bioavailability in the subsurface. In particular, the compound-specific properties of solubility and the organic carbon partition coefficient (K_{oc}) help establish the relative bioavailability of contaminants. These properties can be used to help determine the susceptibility of the contaminant mass to enhanced microbial degradation and, ultimately, the potential effectiveness of enhanced aerobic bioremediation. Note that some compounds (e.g., MTBE) may be relatively bioavailable, but are difficult to biodegrade. Special considerations for MTBE are discussed beginning on page XII-39. This section continues with a discussion of the parameters of solubility and K_{oc} and their influence on enhanced aerobic bioremediation effectiveness.

Solubility. Solubility is the maximum concentration of a chemical that can be dissolved in water at a given temperature without forming a separate chemical phase on the water (i.e., free product). Most petroleum compounds have relatively low solubility values, thus limiting the concentrations of contamination that can be dissolved in groundwater and limiting their bioavailability in the aqueous phase. This is because less contaminant mass is able to reside in groundwater for biodegradation relative to contaminants with higher solubility limits. However, the solubility values for petroleum hydrocarbons range significantly – over four orders of magnitude – as shown in Exhibit XII-16. The solubility values in Exhibit XII-16 represent those of pure phase chemicals. For example, benzene dissolved in water by itself (with no other compounds present) can reach a maximum concentration in water of about 1.79 g/L before a separate phase develops. When multiple compounds are present such as at a petroleum release site, effective solubility values can be expected to be lower. While not representing effective solubility concentrations that may exist at particular petroleum release sites, the values present in Exhibit XII-16 provide a sense for the relative solubility concentrations for a range of fuel components. It is beyond the scope of this document to describe the chemistry involved and how effective solubility might be estimated.

Exhibit XII-16
Solubility Values And Organic Partition Coefficients For Select Petroleum Hydrocarbon Constituents

Compound	Molecular Weight (g/mol)	Solubility in Water (g/L)	Organic Carbon Coefficient (K_{oc} in mL/g)
MTBE	88.15	51	12
Benzene	78	1.79	58
Toluene	92.15	0.53	130
Ethylbenzene	106.17	0.21	220
Xylenes (total)	106	0.175	350
Cumene	120.19	50	2,800
Naphthalene	128	0.031	950
Acenaphthene	154	.0035	4,900

Compounds with higher solubility values are generally smaller, lower molecular weight molecules (e.g., benzene). When spilled, these compounds exist in groundwater at higher relative concentrations and move more quickly through the aquifer than do compounds of higher molecular weights. These compounds are generally more biodegradable because of both their relatively smaller size and bioavailability in the aqueous phase, because proportionately more contaminant mass is in the groundwater where it may be mineralized by aerobic bacteria.

Larger and higher molecular weight hydrocarbon molecules are generally less soluble in water; therefore, their dissolved concentrations in groundwater tend to be limited (e.g., acenaphthene). This property not only reduces the availability of these hydrocarbons to biodegradation, it also limits the mass of these contaminants that can migrate with groundwater over time. For bioremediation of higher molecular weight compounds at a particular site, these two factors may offset one another. In simpler terms, bioremediation of the larger hydrocarbons may take longer, but there is more time to complete the biodegradation because the contamination is not moving away from the treatment area as quickly. The most appropriate remediation for sites that are contaminated mostly with heavy petroleum constituents might be excavation and application of an off-site remedial technology, such as thermal desorption, or proper disposal of the contaminated soil.

Solubility is also an indicator of likely contaminant sorption onto soil. When contaminants are sorbed onto soil particles, they are less available for bioremediation. A compound with a relatively high solubility has a reduced tendency to sorb to soil contacting contaminated groundwater. Conversely, contaminants with relatively low solubility values will generally have an increased tendency to sorb to soil contacting contaminated groundwater. This concept is described in more detail below.

K_{oc} Factor. When groundwater is contaminated by a release from a petroleum underground storage tank, the proportion of hydrocarbon mass in the soil is often far greater than that dissolved in groundwater. This is due in part to the relatively low solubility thresholds for petroleum contaminants. However, another factor is the relatively strong tendency for most petroleum hydrocarbons to sorb to naturally occurring organic carbon material in the soils. This tendency, along with the sheer mass of soil relative to groundwater in a contaminated area, can lead to hydrocarbon mass distributions that are so lopsided they can make the mass in the dissolved-phase appear insignificant. However, because bioremediation occurs in the dissolved phase, that portion of a petroleum mass is always significant in a bioremediation project. It is important to also know how the target organic petroleum compounds are partitioned between the dissolved and unsaturated and saturated sorbed phases.

K_{oc} is a compound-specific property that helps define the equilibrium condition between organic carbon and the contaminant concentrations in an aqueous solution. Using site-specific soil organic carbon content data (i.e., fraction of organic content or foc), K_{oc} can be used to determine the equilibrium contaminant concentrations between groundwater and soil below the water table. The typical organic carbon content in surface soils ranges from 1 to 3.5 percent. In subsurface soils, organic carbon content is an order of magnitude lower because most organic residues are either incorporated or deposited on the surface.

The equation below shows how K_{oc} is defined and used with site-specific fraction of organic carbon (f_{oc}) data to determine the soil-to-groundwater concentration equilibrium ratio, K_d. Knowing the contaminant concentration in one media (e.g., groundwater), the contaminant concentration in the other media (e.g., soil) can be predicted using the site- and constituent-specific K_d sorption constant.

$$K_d = f_{oc} \text{ x } K_{oc}$$

where:

K_d = grams contaminant sorbed/grams organic carbon
= grams contaminant/mL solution
K_{oc} = compound-specific sorption constant and
f_{oc} = fraction of organic carbon in site soil

Higher K_{oc} and K_d values indicate more contaminant mass is likely to be retained in soil and therefore less readily bioavailable. Conversely, lower K_{oc} and K_d values indicate lower contaminant concentrations will exist in equilibrium in soil for given concentrations in groundwater. Exhibit XII-16 provides petroleum constituent K_{oc} values for a list of common petroleum hydrocarbon. A comparison of the solubility and K_{oc} values for the sample group of petroleum hydrocarbons reveals the inverse relationship between the two parameters. For example, compounds with higher solubility values have lower K_{oc} constants.

The relative proportions of contaminants in the sorbed and dissolved phases is important to establish when evaluating the likely effectiveness of enhanced aerobic bioremediation. A disproportionate amount of target hydrocarbon contaminant mass sorbed to the soil, and therefore less bioavailable, may signal that enhanced aerobic bioremediation by itself may not be an effective method of reducing subsurface contaminant mass. In this case, it may be necessary to combine enhanced aerobic bioremediation with other technologies that can help bring more contaminant mass out of the sorbed phase and into the dissolved phase so it can be biodegraded. This highlights the importance of establishing a cleanup goal up front.

In the absence of site-specific data that reveal the distribution of contaminant mass, solubility and K_{oc} data can be used to obtain a general understanding of the likelihood that enhanced aerobic bioremediation is applicable at the site. Petroleum contaminants with generally high solubility limits and low K_{oc} values tend to be more bioavailable in groundwater, and the contaminant mass can often be destroyed by enhanced aerobic bioremediation technologies. When contaminant solubility constants are generally low and K_{oc} values are high, enhanced aerobic bioremediation will be limited in its effectiveness.

Special Considerations for MTBE. Not all sites have indigenous microbial suites capable of degrading MTBE. The MTBE chemical bonds are strong and not easily cleaved through chemical or biological means. As such, when enhanced aerobic bioremediation is to be utilized for addressing MTBE, it may be prudent to verify that native MTBE-degraders exist at a site, before implementing a costly and complex enhanced aerobic bioremediation plan. This can be done with standard microcosm tests. Such laboratory test can be also used to optimize the Enhanced aerobic bioremediation procedures for the site so as to insure enhanced biodegradation of both petroleum compounds and MTBE. If the microcosm tests indicate that insufficient MTBE-degrading microbes exist at a site, then it may be necessary to bioaugment the site by increasing the numbers of microbes. Caution is necessary when bioaugmenting with a cultured microbial suite as the technical effectiveness, cost-effectiveness, and longevity of microbes need to be well understood. Due to the vagaries of geochemistry and microbiology in the subsurface, site-specific microcosms and/or pilot tests may be advisable before full-scale implementation of a bioaugmentation system.

When MTBE biodegrades, it often produces an intermediary product called tertiary butyl alcohol (TBA). The subsurface creation of TBA has been noted at some enhanced aerobic bioremediation field sites that contain MTBE. Therefore, any enhanced aerobic bioremediation application at a site containing MTBE has the potential to create TBA. This constituent of concern has been noted to rapidly disappear from the subsurface at some biodegradation sites, while at other sites, the TBA seems to be recalcitrant. Field workers need to be aware of the possible subsurface creation of TBA, and seek to avoid creating a undesirable, recalcitrant TBA plume.

The presence of TBA in the subsurface at an MTBE-impacted site is not definitive proof of MTBE biodegradation. TBA is a gasoline additive that can be present in concentrations of up to 9.5% by volume, and it is often found in commercial-grade MTBE at 1-2% by volume. Therefore, it is possible to detect subsurface TBA at an MTBE site, even if no MTBE biodegradation is occurring. Careful study of TBA/MTBE ratios, as well as their plume patterns relative to each other and relative to the enhanced aerobic bioremediation activitie,s can help to determine if the TBA was in the original gasoline spill or if it is present due to biodegradation of TBA. It is also important to note that as an alcohol, TBA can be difficult to detect at low levels in water samples; detection limits from laboratory analyses can vary widely, and many analyses will not find TBA when it is present in low concentrations.

When considering enhanced aerobic bioremediation for a site that also contains the gasoline additive methyl tertiary butyl ether, the presence of MTBE mandates that several issues be considered. Exhibit XII-17 provides a list of the questions that should be asked before enhanced aerobic bioremediation is considered for treating MTBE at a petroleum UST site.

EXHIBIT XII-17
MTBE Considerations For Applying Enhanced Aerobic Bioremediation

Does the presence of MTBE require treating a larger region of the aquifer?

Does the presence of MTBE require treating a deeper portion of the aquifer, especially in the downgradient area of the plume where MTBE plumes sometimes “dive” ?

Does either of these mandates require installing more oxygen application points?

Are native MTBE-degrading microbes known to exist at that specific site? Are they sufficient in number to be effective? Are they located where the MTBE presently is? Are they located where the MTBE will be in the future?

Is the addition of an MTBE-degrading microbial suite needed?

Has the greater mobility of the MTBE been accounted for in the plan?

Does the presence of more readily biodegradable compounds (example: BTEX) indicate a delay before MTBE is consumed by microbial populations? If so, what are the implications of this?

Is the same remediation method being used for the hydrocarbons also sufficient to address the MTBE? Does the site contain a sufficient oxygen load and appropriate microbial suite (native or bioaugmented)?

Has the corrective action plan accounted for the possible biological formation of the intermediary product tertial butyl alcohol (TBA), including the possibility of creating an undesirable TBA plume?

Has the corrective action plan accounted for the possible biological formation of the intermediary product tertial butyl alcohol (TBA), including the possibility of creating an undesirable TBA plume?

The various technical issues raised in Exhibit XII-17 demonstrate that while enhanced aerobic bioremediation for MTBE and other similar oxygenates can be promising, a number of special factors should be considered before moving forward with application of an enhanced aerobic bioremediation project for MTBE. Although the addition of supplemental microbial suites (bioaugmentation) is beyond the scope of this chapter, it can be considered for such sites. For more information on the use of bioaugmentation, see *How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers* (US EPA 510-R-04-002), Chapter X (“In-Situ Groundwater Bioremediation”).

As discussed earlier, assessing the applicability of an enhanced aerobic bioremediation plan for MTBE is more complex than a similar assessment for other gasoline compounds. While typical gasoline compounds like BTEX have been found to be nearly ubiquitously biodegradable under a wide variety of subsurface conditions, the same cannot be said for MTBE. Studies of MTBE biodegradability have produced highly variable results.. Therefore, it is not yet possible to make universal statements about enhanced aerobic bioremediation effectiveness for MTBE. Instead, the reviewer is advised to carefully consider site-specific conditions before committing to enhanced aerobic bioremediation for MTBE. Exhibit XII-18 on the next page provides some guidance.

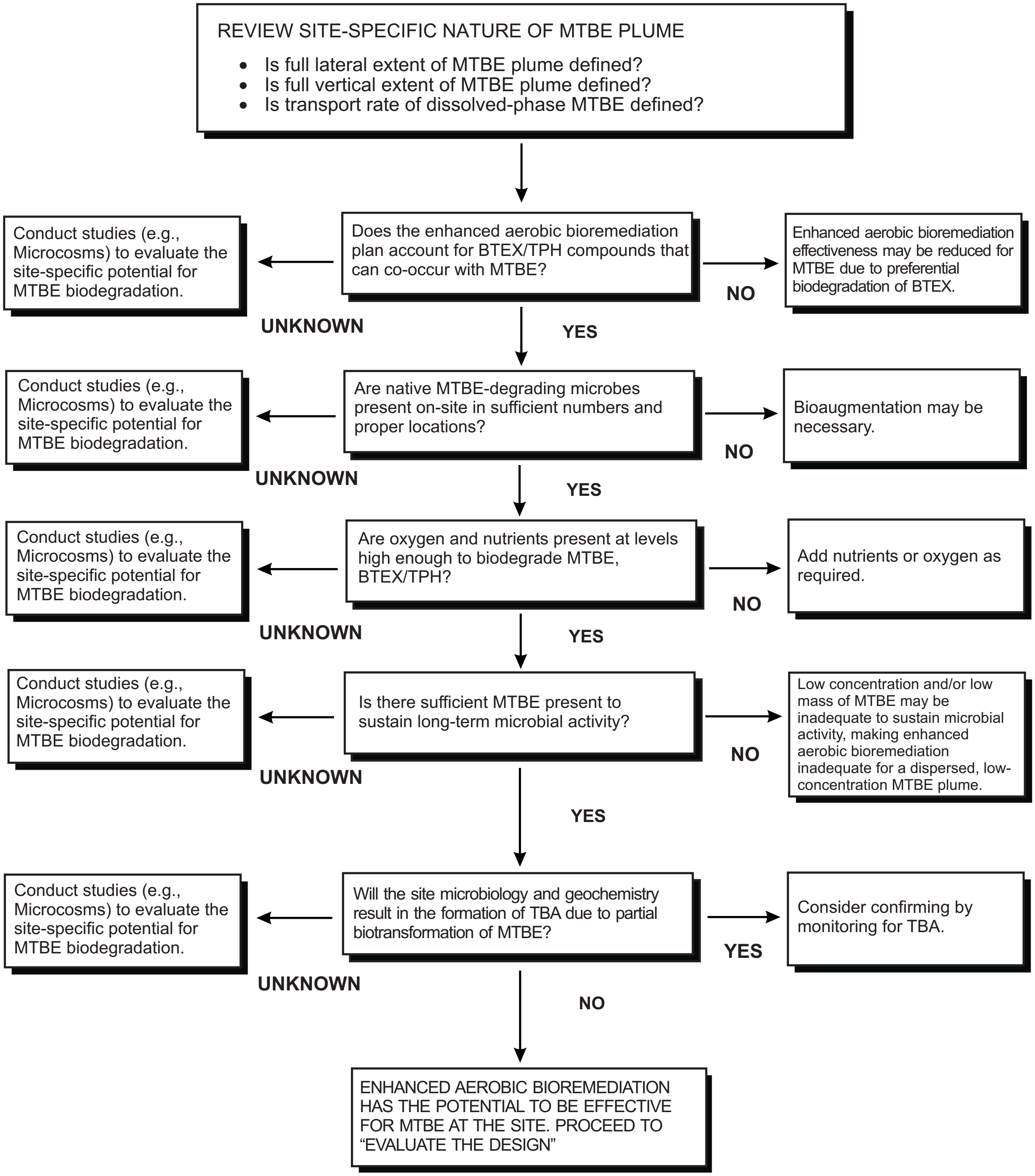
Because MTBE biodegradability still appears to be site-specific and because the state of knowledge is still developing, it may be advisable to conduct site-specific microcosm studies using the intended enhanced aerobic bioremediation method before committing to a full-scale remediation plan for MTBE. Such microcosm studies may investigate: MTBE biodegradation under varying conditions, the need for bioaugmentation, the production of TBA, etc.

Step 3 - Evaluation of Enhanced Aerobic Bioremediation Design

This section provides guidance on reviewing and evaluating the enhanced aerobic bioremediation design. It focuses on prompting reviewers to identify and review key elements of corrective action plans to help ensure they demonstrate a coherent understanding of the basis for the enhanced aerobic bioremediation system design**.** In addition, this section provides information on typical enhanced aerobic bioremediation technology components to help verify that the corrective action plan has included the basic equipment requirements for the remedial system.

It is assumed that the detailed technology screening process (described in Steps 1 and 2) has verified that enhanced aerobic bioremediation appears to be appropriate and is expected to be an effective cleanup approach, given site-specific conditions. If the enhanced aerobic bioremediation effectiveness evaluation has not been completed, it is strongly recommended that this be done before the design is evaluated.

Exhibit XII-18
Detailed Evaluation of Enhanced Aerobic Bioremediation Effectiveness for MTBE



Design Basis

Review of the corrective action plan should find consistency between site characterization work and information that is presented as the basis for the enhanced aerobic bioremediation design in the corrective action plan. To conduct the enhanced aerobic bioremediation effectiveness evaluation, the reviewer should have a solid understanding of the nature and extent of the site-specific petroleum constituents of concern, including an understanding of the contaminant phases present and the relevant site chemical, physical, and biological properties. When preparing and reviewing the corrective action plan design, the reviewer should also understand the site geology and hydrogeology, and the risks associated with the contamination. These data, which should have been developed and interpreted as part of the site characterization effort, serve as the foundation for the remedial system design.

While the site characterization data provide the core raw materials for the design, further refinement is often needed and useful. For example, while the site characterization work may identify potential human or ecological receptors that may be exposed to the contamination, specific cleanup goals may not have been established. In such cases, the specific remedial goals would need to be developed and identified in the corrective action plan through one or more established approaches, such as adopting state-published cleanup standards, developing site-specific risk-based standards acceptable to the state, or employing other state-specific and approved methods.

The corrective action plan may also include the results and interpretation of follow-up studies completed after the original site characterization. The need for such studies is often identified after a review of the site characterization shows that additional information is needed to complete the remedial system design. For example, the site characterization may suggest that one or more of the constituents of concern is believed to be marginally biodegradable, and the level of expected biodegradation is difficult to predict from the existing data.

Examples of typical information expected to be developed during the site characterization, or as a result of follow-up studies that are completed to support the basis for the technology selection and design of the corrective action plan, are summarized in Exhibit XII-19. Each of the items listed in Exhibit XII-19 is described in more detail below.

Cleanup Goals

The evaluation of alternative remedial approaches and the subsequent design of the selected approach are strongly influenced by the cleanup goals that the remediation program must achieve. Often, preliminary goals identified during the site characterization work evolve as a better understanding of site conditions and potential receptors is attained. However, owing to their importance for remediation planning and design, the cleanup goals should be fully evolved and solidified in the corrective action plan.

Exhibit XII-19
Enhanced Aerobic Bioremediation Design Basis Factors

Design Basis Factor	Source(s) of Design Information
Cleanup Goals # Target contaminant levels (soil and groundwater) # Remediation timeframe	Receptor survey, pre-design exposure or risk assessment analyses (potentially including numerical modeling), or state requirements
Geology # Uniformity # Stratigraphy # Geochemistry # Bedrock # Soil permeabilities	Site characterization soil borings, well installations, sampling/analysis, and site observations. Local geologic studies.
Hydrogeology # Depth to groundwater # Groundwater elevation and gradient # Aquifer/water bearing unit class (e.g, confined, unconfined, perched, bedrock) # Hydraulic parameters (e.g., conductivity, transmissivity, storativity, effective porosity) # Modeling results	Site characterization well gauging, aquifer pump testing, data analyses, and local hydrogeologic studies.
Petroleum Contamination # Target chemical constituents # Target contaminant and total hydrocarbon mass estimates (sorbed, dissolved, liquid and vapor phases) # Extent (vertical and lateral) # Bioavailability # Biodegradability # Fate and transport characteristics	Soil, groundwater and other media sampling/laboratory analysis, review of published data on contaminants and data interpolation and analysis.

Cleanup goals usually provide the end-point concentrations for petroleum constituents in soil and groundwater that are acceptable to state or other regulatory agencies. These cleanup thresholds could be goals that represent any of the following:

- # Health-based numeric values for petroleum chemical constituents published by the respective regulatory agency
- # Cleanup goals developed and proposed by the contractor specifically

for the contaminated site that are acceptable to the Implementing Agency

- # Goals derived from site-specific risk assessment involving contaminant fate and transport modeling coupled with ecological and human-health risk assessment
- # Generic state cleanup goals

Additional project goals that may or may not be regulatory requirements include hydraulic control of the contamination, a cleanup timeframe, or other performance goals established in the corrective action plan. Regardless of what the cleanup goals are and how they are established, the state-sanctioned goals should noted in the corrective action plan and recognized as a *fundamental* basis for the technology selection and design.

The cleanup goals presented in the corrective action plan answer important questions relevant to the viability of the selected remedial approach and the adequacy of the remedial design. These two critical questions are:

- # Can the cleanup concentration goals be met by the designed enhanced aerobic bioremediation system?
- # Can sufficient oxygen be delivered to the contaminated area to enable contaminants to be biodegraded to meet cleanup goals within a reasonable period of time?

Each of these questions is discussed in more detail in the paragraphs that follow.

- # *Can the cleanup concentration goals be met by the designed enhanced aerobic bioremediation system?*

Below a certain “threshold” petroleum constituent concentration, bacteria may not be able to derive sufficient carbon from petroleum biodegradation to sustain vigorous levels of biological activity. As concentrations of petroleum contaminants fall below the threshold, further biodegradation of the petroleum hydrocarbons can become relatively insignificant. The level of diminishing returns is site-specific and representative of petroleum contamination that has been reduced in concentration to the technological limit of the specific enhanced aerobic bioremediation.

Although the threshold limit of enhanced aerobic bioremediation approaches can vary greatly, depending on bacteria-, petroleum constituent- and site-specific factors, it is generally observed that petroleum constituent soil concentrations cannot be reduced below 0.1 ppm without using supplemental technologies. In addition, reductions in total petroleum hydrocarbons (TPH) of greater than 95 percent can be very difficult to achieve because of petroleum products often contain “recalcitrant” or non-degradable petroleum hydrocarbons.

While further bioremediation of petroleum contaminant levels in the subsurface may become limited at some point due to the limited availability of a useable carbon source, it is quite possible that the target chemical constituents that may exist in soil and groundwater at that time may meet the cleanup standards. Even though total hydrocarbon levels may remain elevated in subsurface soil, the chemical constituents comprising the hydrocarbon mass may be those that are less soluble and of reduced environmental concern.

Exhibit XII-20
Cleanup Concentrations Potentially Achieved By Enhanced Aerobic Bioremediation

Cleanup Requirement	Feasibility of Meeting Cleanup Levels
Petroleum constituent concentration in soil >0.1 ppm (each contaminant with corresponding dissolved levels in groundwater) and TPH reduction < 95%	Feasible
Constituent concentration in soil $\leq$ 0.1 ppm (each contaminant with corresponding dissolved levels in groundwater) or TPH reduction $\geq$ 95%	Potentially infeasible to remediate in reasonable timeframe; laboratory or field trials may be needed to demonstrate petroleum concentration reduction potential

If comparing existing levels of site petroleum contamination to the cleanup goals indicates that either of these guidance criteria summarized in Exhibit XII-20 is exceeded, the proposed enhanced aerobic bioremediation. The system design may not achieve the expected remedial objectives in a reasonable time frame.

*Can sufficient oxygen be delivered to the contaminated area to enable contaminants to be biodegraded to meet cleanup goals within a reasonable period of time?*

Cleanup goals establish the concentrations and allowable residual mass of petroleum constituents that can acceptably remain in the subsurface soil and groundwater subsequent to remediation. The difference between the current level of petroleum mass in the soil and groundwater and the allowable residual mass left in the subsurface is the mass that needs to be biodegraded using enhanced aerobic bioremediation. Using the theoretical 3 to 3.5 pounds of O_2 to degrade roughly 1 pound of petroleum hydrocarbon ratio discussed earlier, it is possible to estimate the minimum mass of O_2 needed to achieve the required petroleum mass biodegradation. This value assumes that there are no significant oxygen "sinks" in the subsurface (e.g., mineral species that oxidize such as iron) that would increase the total demand for oxygen.

For example, if the corrective action plan data indicate that approximately 5,000 pounds of petroleum hydrocarbons are in the site subsurface but the cleanup goals allow only 500 pounds to remain after remediation (based on allowable soil and groundwater constituent concentration limits), then 4,500 pounds of hydrocarbons require bioremediation. Assuming anaerobic biodegradation and abiotic degradation of site contamination are negligible, and that there are no other sources of oxygen or significant oxygen losses or sinks, and 3.5 pounds of O_2 are needed to aerobically biodegrade each pound of petroleum, then it can be estimated that a minimum of 15,750 pounds of oxygen would need to be provided by the enhanced aerobic bioremediation technology during remedial program implementation. During review of the corrective action plan, therefore, estimate the oxygen mass required to bioremediate the contamination and determine how the demand will be met by the proposed enhanced aerobic bioremediation system.

Furthermore, if pure oxygen injection is the proposed enhanced aerobic bioremediation technology, and the remediation timeframe is 3 years, the corrective action plan design should show how the pure oxygen injection system will be able to deliver and distribute a minimum of 15,750 pounds of oxygen over the 3-year period. In other words, the corrective action plan should demonstrate that an average of at least 0.6 pounds of pure oxygen per hour can be delivered over the 3-year period.

The example discussed above assumes that losses of oxygen to the aquifer are negligible. In reality, as discussed earlier in this chapter, significant losses of oxygen can occur from the application of the enhanced aerobic technology itself and from abiotic and microbiologically mediated reactions with the aquifer material. An attempt should be made to estimate what these potential oxygen losses could be in order to factor those losses into the oxygen delivery plan and cleanup schedule.

If the corrective action plan does not estimate the oxygen and bio-nutrient delivery requirements or does not demonstrate how the oxygen and bio-nutrient delivery requirements will be met by the enhanced aerobic bioremediation system, the corrective action plan may be incomplete. Under such circumstances, it may be prudent to request that this information be provided before approving the plan. Similarly, if site-specific cleanup goals have not been clearly established in the corrective action plan or previously, it may be appropriate to refrain from completing the review of the design until this critical information is provided.

Enhanced Aerobic Bioremediation Technology Selection

With the design basis established in the corrective action plan, the corrective action plan can be reviewed to confirm that enhanced aerobic bioremediation is a reasonable site-specific choice of remediation technology. Depending on project-specific circumstances, there can be only one or a few enhanced aerobic bioremediation technologies equally viable and appropriate for a site.

Alternatively, site-specific or project-specific circumstances may suggest that one of the enhanced aerobic bioremediation would address the on-site contamination better than any other technology.

Exhibit XII-2 presents the key advantages and disadvantages of each of the enhanced aerobic bioremediation technologies. Use these factors to evaluate the feasibility of using an enhanced aerobic bioremediation approach. Other differences between and among alternative enhanced aerobic bioremediation technologies can help to distinguish their most appropriate application(s). A key characteristic useful for evaluating the feasibility and appropriateness of a proposed enhanced aerobic bioremediation technology is oxygen delivery efficiency. More information on how this characteristic can be used is provided in the next paragraphs.

Oxygen Delivery Efficiency. All enhanced aerobic bioremediation technologies need to deliver oxygen to the subsurface to encourage aerobic biodegradation of petroleum contamination to occur. The effectiveness of each enhanced aerobic bioremediation technology is directly related to the amount of oxygen it can deliver and uniformly distribute in the contaminated area. Because of this commonality, it makes sense to explore the relative efficiency with which each technology is able to deliver oxygen to the treatment area as a distinguishing feature.

Oxygen produced from the decomposition of compounds used in enhanced aerobic bioremediation approaches follows the stoichiometric relationships shown in Exhibit XII-21. For instance, for every two parts of hydrogen peroxide injected, only one part of oxygen is produced. In contrast, one part ozone yields 1.5 parts of oxygen, a seemingly more efficient means of generating oxygen.

Exhibit XII-21
Basic Stoichiometry Oxygen Production From Chemical Decomposition

Enhanced Aerobic Bioremediation Technology	Basic Oxygen-Producing Stoichiometry
Oxygen-Producing Compounds	
Hydrogen Peroxide	$2H_2O_2 \rightarrow 2H_2O + O_2$
Ozone	$O_3 \rightarrow 1.5\ O_2$
Oxygen Releasing Compounds	
Magnesium Peroxide	$MgO_2 + H_2O \rightarrow Mg(OH)_2 + \frac{1}{2}O_2$
Sodium Peroxide	$Na_2O_2 + H_2O \rightarrow NaOH + H_2O_2$

A more practical way of measuring oxygen delivery efficiency is to determine the total amount of mass of carrier material (e.g., groundwater containing hydrogen peroxide) that needs to be delivered to the subsurface in order to deliver 1 gram of oxygen. In essence, this is a measure of the amount of effort, energy, and perhaps, time required to deliver oxygen using the different enhanced aerobic bioremediation technologies. Exhibit XII-22 compares seven alternative methods of delivering oxygen to the subsurface using this measure of delivery efficiency. It compares:

- Three approaches that use groundwater as the oxygen carrier
  - Re-injection of groundwater fully aerated with ambient air
  - Re-injection of groundwater fully aerated with pure oxygen
  - Re-injection of groundwater containing 100 ppm of hydrogen peroxide

- One method that delivers oxygen in the solid phase (oxygen releasing compounds)

- Three approaches that deliver oxygen in the vapor phase
  - Ozone injection
  - Biosparging/bioventing
  - Pure oxygen injection

While the re-infiltration of hydrogen peroxide-amended groundwater may be the least efficient method of oxygen delivery to the contaminated area, the hydraulic gradients induced by this activity may enhance the distribution of oxygen in the subsurface. For more information on factors affecting the distribution of oxygen in the subsurface, refer to discussions presented earlier as part of the detailed enhanced aerobic bioremediation effectiveness evaluation.
Each of the major headings in the table above is discussed in more detail below.

Design Components

Although the design elements of alternative enhanced aerobic bioremediation technologies can vary significantly, Exhibit XII-23 describes the most common design elements. Several of the more important elements are discussed below to assist with evaluation of the corrective action plan.

Oxygen and Bio-nutrient Delivery Design should be based primarily on petroleum mass reduction requirements, site characteristics and cleanup goals. Oxygen will generally need to be applied at a minimum 3:1 ratio relative to the petroleum hydrocarbon mass targeted for remediation. Bio-nutrient formulation and delivery rate (if needed) will be based on soil sampling. Common nutrient additions include nitrogen (in an aqueous solution containing ammonium ions) and phosphorus (in an aqueous solution containing phosphate ions). Note that state regulations may either require permits for nutrient and/or air injection or prohibit them entirely.

Exhibit XII-22
Relative Oxygen Delivery Efficiencies For Various Enhanced Aerobic Bioremediation Technologies

Oxygen Delivery Approach	Description	Oxygen Concentration in Delivery Material (mg/L)	Mass of Oxygen Carrier per Unit Mass of Oxygen Delivered (g/g)	Relative Oxygen Delivery Efficiency
Aqueous-Phase Oxygen Delivery				
Re-injection of Aerated/ treated Groundwater	Ambient Air Saturated	9	110,000	Lowest
Re-injection of Pure Oxygen-Amended Groundwater	Pure O_2 Saturated	45	22,000	
Re-injection of H_2O_2-Amended Groundwater	100 mg/L of H_2O_2	50	20,000	
Solid-Phase Oxygen Delivery				
Injection of Oxygen-Releasing Compounds	Mg-peroxide	N/A	10	
Vapor Phase Oxygen Delivery				
Injection of Ozone	5% Ozone (Converted to O_2)	98	12	
Biosparging with Air or Oxygen, or Bioventing	21% Oxygen (Ambient)	275	4	
Injection of Pure Oxygen	95% Oxygen	1,250	1	Highest

Exhibit XII-23
Common Enhanced Aeration Remediation Design Elements

Oxygen and Bio-nutrient Delivery Design
- Theoretical oxygen mass requirement
- Bio-nutrient needs (e.g., N, P)
- Application delivery rate
- Number and depth of application points/position
- Equipment

Permit Requirements and Thresholds
- Underground injection/well installation
- Air injection into subsurface
- Groundwater (wastewater) discharge
- Air (soil vapor) discharge

Performance Monitoring Plan
- Ongoing distribution of oxygen and bio-nutrients
- Expansion of microbial population
- Reduction in contaminants (sorbed and dissolved phases)

Contingency Plan
- Inadequate oxygen distribution
- Stagnation or die-off of microbial population
- Lower-than-expected petroleum mass reduction rates
- Excessive contaminant migration
- Build-up of excessive recalcitrant petroleum constituents
- Fugitive (soil vapor) emissions
- Difficult-to-treat/fouling of treated wastewater discharge
- Clogging of equipment or injection areas with iron oxide or biomass
- Other contingencies

Permit Requirements and Thresholds should be identified in the design so that the system can be constructed to comply with permit requirements and constraints. Depending on the specific enhanced aerobic bioremediation technology and the state in which the site is located, permits that may be required include underground injection, treated groundwater discharge (to sanitary or storm sewer, or air (soil vapor) discharge.

Several federal, state, and local programs regulate Class V aquifer remediation wells, and many require permits for underground injection of oxygen or bio-nutrients. On the federal level, management and regulation of these wells fall primarily under the underground injection control program authorized by the Safe Drinking Water Act (SDWA). Some states and localities have used these authorities, as well as their own authorities, to extend the controls in their areas to address concerns associated with aquifer remediation wells. Aquifer remediation injection wells are potentially subject to at least three categories of regulation.

First, a state's underground injection control (UIC) program, operating with approval from the federal program, may have jurisdiction over such wells. Second, in some states without UIC programs, the state's program for groundwater protection or pollution elimination program requirements may apply to remediation wells. Third, remediation wells may be regulated by federal and state authorities, through Superfund programs, corrective action programs under RCRA (including the UST program), or other environmental remediation programs. In the case of remediation programs, the regulatory requirements typically address the selection of aquifer remediation as a cleanup alternative and establish the degree of required cleanup in soil and groundwater, while deferring regulation of the injection wells used in the remediation to other programs. In the case of voluntary cleanup programs, some concern exists because they may not be approved or completed according to standards typical of cleanups overseen by a state or federal agency.[4]

Performance Monitoring should be accounted for in the design in the form of a written plan that can be used to objectively evaluate enhanced aerobic bioremediation system performance. The plan should clearly describe the approaches and methods that will be used to evaluate enhanced aerobic bioremediation system effectiveness in each of the following:

- Delivering oxygen (and bio-nutrients) to the subsurface
- Distributing oxygen and bio-nutrients through the contaminated area
- Increasing microbial population density
- Reducing sorbed and dissolved phase petroleum concentrations
- Achieving other performance requirements consistent with site-specific cleanup goals

Contingency Plans should also be accounted for and prepared as part of the design. The design should anticipate low-likelihood problems and potentially changing environmental conditions, as well as outline specific response actions that may be taken. Examples include response actions to take if any performance monitoring data indicate the following:

- Inadequate oxygen distribution
- Stagnation or die-off of microbial populations
- Low petroleum mass reduction rates
- Excessive contaminant migration

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[4] US EPA, Ofice of Solid Waste memo dated 12/27/00 on the Applicability of RCRA Seciton 3020 to In-Situ Treatment of Ground Water.

- # Recalcitrance of constituents
- # Fugitive emissions
- # Any other reasonably plausible scenario that can arise under site-specific conditions and project-specific circumstances.

Components of Enhanced Aerobic Bioremediation Systems

After review of factors that affect the selection and design of a particular enhanced aerobic bioremediation technology and the critical elements that should be included in the corrective action plan for enhanced aerobic bioremediation,, it is now appropriate to discuss major components of various enhanced aerobic bioremediation systems.

Exhibit XII-24 summarizes some of the major equipment components associated with each of the more common enhanced aerobic bioremediation technologies. Depending on which enhanced aerobic bioremediation technology has been selected in the corrective action plan, a subset of these major system components should be presented and discussed and schematically depicted (e.g., process flow diagram) in the corrective action plan. The design should relate capacities of these equipment components to design requirements (e.g., required oxygen production/delivery rates).

As shown in Exhibit XII-24, enhanced aerobic bioremediation systems employing oxygen-releasing compounds appear to require the least equipment in part because there is no need for any mechanical equipment once the oxygen-releasing compounds are deployed. By contrast, re-injection of hydrogen peroxide-amended groundwater requires the most equipment and a large number of mechanical components (e.g., pumps, blowers, etc.).

While the sets of major equipment components used by the enhanced aerobic bioremediation technologies differ significantly, the use of wells by each different approach warrants recognition and further discussion. In particular, the orientation, placement, number and construction of this common design element is worthy of a brief review.

Injection, Extraction and Re-infiltration Wells. Three important considerations for these wells are orientation, placement and number, and construction.

- # **Well Orientation.** Both horizontal and vertical wells can be used to treat subsurface petroleum releases with any of the various enhanced aerobic bioremediation systems. Hydrogen peroxide-amended groundwater can be re-infiltrated using either vertical or horizontal wells. Although vertical wells are more common for ozone or pure oxygen injection, horizontal wells can be used.

Exhibit XII–24
Major Components of Enhanced Aerobic Bioremediation Systems

Component	Function
Oxygen Releasing Compound Systems	
Borings and Excavations	Used to inject or place a slurry of oxygen releasing compounds so that oxygen may be slowly imparted to the water bearing zone.
Application Wells	Often used to suspend a solid form of oxygen releasing compounds to provide oxygen to groundwater.
Monitoring Wells	Used to evaluate effectiveness of remedial approach. Comparative analyses over time of groundwater samples from these wells for dissolved oxygen and petroleum contamination generally indicate how effectively oxygen is being delivered/dispersed and contaminants are being reduced.
Hydrogen Peroxide Injection Systems	
Extraction Wells	Often used to extract contaminated groundwater downgradient of the contaminated area for treatment and re-injection in the upgradient source area for plume containment and/or accelerated groundwater flow through the contaminated area.
Injection Wells or Infiltration Galleries	Injection wells, infiltration galleries or a combination of these are typically used to re-inject treated and hydrogen peroxide-amended groundwater so that dissolved oxygen may be flushed through the treatment zone.
Extraction, Injection, Transfer, and Metering Pumps and Tanks	Extraction, injection, transfer, and metering pumps are used for various purposes including: transferring groundwater from and back into the ground; transferring extracted groundwater between different components of the treatment system; and metering hydrogen peroxide and bio-nutrients into the infiltration system to maintain design concentrations.
Groundwater Treatment Equipment	Extracted groundwater may be treated to remove petroleum hydrocarbons by various means such as: oil/water separation; air stripping; or granular activated carbon sorption or others.
Instrumentation and Controls	Used to integrate and activate/deactivate system components. Help maintain the balance of flows consistent with the design and to safeguard against inadequate treatment or inappropriate discharges.

Exhibit XII–24
Major Components of Enhanced Aerobic Bioremediation Systems (continued)

Component	Function
Hydrogen Peroxide Injection Systems (continued)	
Monitoring Wells	Used to collect environmental samples analyzed in laboratories and field to evaluate on-going effectiveness of remediation. Groundwater well samples tested for dissolved oxygen and contamination to evaluate overall effectiveness of oxygen delivery/dispersal and the contaminant reductions over time.
Pure Oxygen Injection Systems	
Sparging Wells	Used as conduits to bubble pure oxygen into contaminated groundwater. The oxygen is delivered to the base of the soil and groundwater petroleum contamination so that it will rise through the contaminated material providing oxygen to the hydrocarbon degrading bacteria.
Air Compressing Equipment	Used to pressurize ambient air to: prepare it for subsequent treatment to increase Oxygen levels/purity; and to provide pressure needed to inject oxygen and ambient air beneath the water table.
Oxygen Generating Equipment	Used to generate nearly-pure oxygen gas (~ 95%) from ambient air. Synthetic zeolite sorbers are frequently employed to simply remove nitrogen from ambient air to produce high-purity oxygen.
Instrumentation and Controls	Used to integrate and activate/deactivate system components to maintain the balance of flows consistent with design and to safeguard against inadequate treatment or inappropriate discharges.
Monitoring Wells	Used to collect environmental samples tested in laboratories and the field to evaluate on-going effectiveness of remediation. Comparative analyses over time of groundwater samples from these wells for dissolved oxygen and petroleum contamination generally indicate how effectively oxygen is being delivered or dispersed and contaminant reductions are occurring.

Exhibit XII–24
Major Components of Enhanced Aerobic Bioremediation Systems (continued)

Component	Function
Ozone Injection Systems	
Sparging Wells	Used as a conduit to inject ozone into contaminated groundwater. The ozone is sparged near the base of the soil and groundwater petroleum contamination so that it may contact the contaminants and provide oxygen to the hydrocarbon degrading bacteria.
Air Compressing Equipment	Used to pressurize ambient air needed to generate ozone and to provide the pressure needed to inject the ozone beneath the water table. Air compressor equipment must supply oil and contaminant free air to minimize in-line reactions with and premature decomposition of ozone.
Ozone Generating Equipment	Used to generate ozone gas on-site, typically at concentrations of about 5%.
Soil Vapor Extraction/ Treatment Equipment (Optional)	Used, if necessary, to control fugitive soil vapor ozone and volatilize organic compounds emissions in the unsaturated zone. May consist of low vacuum/flow blower to generate vacuum conditions in unsaturated zone and collect the vapors. Vapor treatment may consist of granular activated carbon or biofilters for low contaminant concentration air stream.
Instrumentation and Controls	Used to integrate and activate/deactivate system components to maintain the balance of flows consistent with the design and to safeguard against inadequate treatment or inappropriate discharges.
Monitoring Wells	Used to collect environmental samples tested in laboratories and the field to evaluate ongoing effectiveness of remediation. Comparative analyses over time of groundwater samples from these wells for dissolved oxygen and petroleum contamination generally indicate how effectively oxygen is being delivered or dispersed and contaminant reductions are occurring.

Well orientation should be based on site-specific needs and conditions. For example, horizontal systems should be considered when evaluating sites that require re-infiltration of amended groundwater into shallow groundwater at relatively high flow rates. They are also readily applicable if the affected area is located under a surface structure (e.g., a building), or if the thickness of the saturated zone is less than 10 feet.

- **Well Placement and Number of Wells**. The number and location of wells are determined during the design to accomplish the basic goals of: (1) optimizing reliable oxygen and bio-nutrient delivery to the contaminated area; and (2) providing conduits to measure enhanced aerobic bioremediation system performance. For hydrogen peroxide re-infiltration systems this typically means placing re-injection wells in the source area(s) while extracting groundwater from downgradient locations aimed at simultaneously providing enhanced hydraulic gradient and accelerated oxygen distribution across the impacted area. The number, location, and design of the extraction wells will largely be determined from site-specific hydrogeology, the depth(s) and thickness(es) of the contaminated area(s), and the results of field-scale pilot testing and hydraulic modeling.

Determining the number and spacing of the wells for ozone or pure oxygen injection may also be determined through field-scale pilot testing. However, the following general points should be considered.

  - Closer well spacing is often appropriate in areas of high contaminant concentrations to enhance contaminant contact and oxygen delivery/distribution where the oxygen demand is the greatest.

  - Direct delivery of oxygen into the contaminated material using closer well spacings can deliver and disperse more quickly than oxygen delivery through groundwater advection/dispersion and could significantly decrease the treatment timeframe.

  - At sites with stratified soils, wells screened in strata with low permeabilities often require closer well spacing than wells screened in strata with higher permeabilities.

- **Well Construction.** Enhanced aerobic bioremediation system wells are generally constructed of one- to six-inch diameter PVC, galvanized steel, or stainless steel pipe. Oxygen or ozone injection sparge wells have screened intervals that are normally one to three feet in length and situated at or below the deepest extent of sorbed contaminants. Injection sparge points must be properly grouted to prevent the injected oxygen from moving directly up the well annulus to the unsaturated zone rather than being forced into the contaminated aquifer (“short circuiting” of the injected oxygen). When horizontal injection wells are used, they should be designed and installed carefully to ensure that the injected oxygen exits along the entire screen length.

Re-infiltration wells typically have screen lengths that extend from the base of the wells into the unsaturated zone. Groundwater extraction wells should ideally be screened in the saturated interval containing the greatest mass of hydrocarbons. Field-scale pilot studies and subsequent data analysis and hydraulic modeling can help to determine the configuration and construction design of groundwater extraction and injection wells.

Step 4 - An Evaluation of the Operation and Monitoring Plan

Remedial Progress Monitoring

Significant uncertainties associated with site conditions can remain even as remedial designs are completed and implemented. In the post-remedial startup period, these unknowns frequently can result in operations that vary from the design. These variances can be small or large and often require adjustments to account for unforeseen conditions and optimize system performance. Unfortunately, in many cases, the need for these adjustments can go unrecognized for a long time.

In some cases, the delay in recognizing that remedial system adjustments are necessary may be attributed to relatively slow responses in subsurface conditions to the applied technology (e.g., increases in microbial population and biodegradation of contaminants). Because these subsurface responses to the applied remedial technology can be delayed, there is often the tendency to give the remedial program more time to work (sometimes up to years) before making system modifications or adjustments. In other cases, the delay may stem from misuse or misinterpretation of site data leading to a belief that the remedial system is performing well when it is not. An example of this misuse is the practice of using groundwater analytical data from oxygen delivery wells as an indicator of remedial progress. In this case, an assessment is biased by the localized effects of bioremediation in the immediate vicinity of the oxygen delivery wells, but does not provide an objective measure of the enhanced aerobic bioremediation system's ability to distribute oxygen and promote biodegradation throughout the treatment area.

Wells that are used to carry out remedial actions should not be used as monitoring wells. Monitoring wells should be separate wells used only for that purpose. If remediation involves injection of gases, the monitoring wells should be tightly capped until used. If they are not capped, the monitoring wells can provide a path of least resistance for the injected air to return to the surface. Air can channel to a monitoring well, then bubble up through the standing water in the well preferentially removing contaminants from the area in and immediately around the well while the rest of the aquifer is short circuited.

However, at many sites remedial system operational efficiencies are not optimized simply because an adequate performance monitoring plan has either not been developed or has not been fully implemented. In such cases, the designed remedial system may be installed, started up, and allowed to run its course with insufficient numbers or types of samples collected to determine whether the remedial system is performing in accordance with design expectations. The result of such monitoring approaches can be the discovery of a sub-standard or failed remediation program years after its implementation.

The previous section discussed the importance of developing a comprehensive remedial progress monitoring plan. Because of its importance, this section covers the topics that should be addressed in such a plan to ensure objective gauging of remedial system performance and necessary optimization adjustments can be made early on and throughout the duration of enhanced aerobic bioremediation. In particular, a focused discussion on performance sampling and enhanced aerobic bioremediation system evaluation criteria is provided to assist with the corrective action plan review.

Evaluation Sampling

Evaluation sampling is performed to gauge the effectiveness of the enhanced aerobic bioremediation system relevant to design expectations. Based on a comparison of the actual field sampling data to design and operational expectations, timely modifications to the system or operating procedures (if any) can be made to optimize system performance early in the remediation program. Projects with regular performance reviews guided by the results of such sampling/monitoring programs have a greater chance of achieving the design remedial goals within desired time frames, potentially at lower cost.

Various environmental media are sampled to evaluate system performance. Groundwater, soil, and soil vapors from the treatment area and vicinity are commonly sampled to determine the degree to which the enhanced aerobic bioremediation system is meeting the basic objectives of the approach, including:

- Delivering oxygen to the saturated zone at required design rates
- Distributing dissolved oxygen across the target contaminated area to restore and maintain aerobic conditions
- Reducing concentrations of petroleum hydrocarbons in soil and groundwater at design rates through biodegradation of the petroleum compounds

Exhibit XII-25 identifies those parameters that are commonly measured in groundwater, soil, and soil vapor samples to help evaluate enhanced aerobic

Exhibit XII-25
Common Performance Monitoring Parameters and Sampling Frequencies

Analytical Parameter	**Sampling Frequency**			**Purpose**
	Startup Phase (7-10 days)	**Remediation/Post-Application Long-Term Monitoring Phase (on-going)**		
	Daily	***Weekly to Monthly***	***Quarterly to Annually***	
Groundwater				
Dissolved Oxygen	X	X		Determines system's effectiveness in distributing oxygen and ability to maintain aerobic conditions (i.e., dissolved oxygen > 2 ppm) in treatment area. Provides data to optimize system performance.
Redox Potential	X	X		Yields data on system's ability to increase the extent of aerobic subsurface environment.
pH	X	X		Confirms pH conditions are stable and suitable for microbial bioremediation or identifies trends of concern.
H_2O_2 or Ozone	X	X		Provides information on distances these oxygen-producing compounds can be transmitted by the remedial system before decomposing
Bio-nutrients			X	Determines if bio-nutrients injected into the groundwater are being consumed during bioremediation or accumulating and potentially degrading groundwater quality
Petroleum COCs			X	Indicates remedial progress

Exhibit XII-25
Common Performance Monitoring Parameters and Sampling Frequencies (continued)

Analytical Parameter	Sampling Frequency			Purpose
	Startup Phase (7-10 days)	Remediation/Post-Application Long-Term Monitoring Phase (on-going)		
	Daily	*Weekly to Monthly*	*Quarterly to Annually*	
Groundwater (continued)				
Degradation Daughter Constituents (e.g., TBA)			X	Offer direct evidence of contaminant bioremediation and enhanced aerobic bioremediation effectiveness
Water Table Elevations	X	X		Determines if hydraulic conditions (groundwater flow) are consistent with design intent or if enhanced aerobic bioremediation technology application has had an unanticipated affect on these conditions
Soil Vapor				
Carbon dioxide	X	X		Provides evidence of biodegradation
Oxygen	X	X		Indicates potential losses of introduced oxygen through the unsaturated zone
Volatile Petroleum COCs	X	X		Suggests residual sources in soil or fugitive emissions associated with the remedial effort
Fugitive Ozone or Hydrogen Peroxide	X	X		Determines losses of oxygen-yielding reagents delivered to the subsurface
Soil				
Petroleum COCs			X	Provide a measure of remedial progress and the extent to which biodegradation of sorbed contaminants is limited by

bioremediation progress and system performance. A brief description of the respective sampling frequencies and the relevance and significance of each parameter to the performance evaluation are also provided in the exhibit. A key element is the location(s) where performance evaluation sampling takes place relative to subsurface oxygen delivery points. As stated in the exhibit, performance evaluation samples should not normally be collected from oxygen delivery locations.

The performance of the enhanced aerobic bioremediation system should be determined by the chemistry and microbiology of soil and groundwater located between, around, and downgradient of oxygen delivery locations rather than inside or in the immediate vicinity of the oxygen delivery points. Conditions inside or in the immediate vicinity of oxygen injection locations have been preferentially altered by enhanced aerobic bioremediation to enhance biodegradation of the petroleum contaminants. Therefore, data from these locations are not representative of the subsurface conditions that exist beneath most of the site. To understand the effect the enhanced aerobic bioremediation system is having on the subsurface conditions as a measure of its performance, samples of soil, groundwater and soil gas should be collected from alternate locations.

In reviewing of the performance monitoring plan in the corrective action plan, a reviewer should verify that a sufficient number of sampling locations exist between oxygen application points to provide the necessary performance sampling data. A description of how these data may be used to evaluate the enhanced aerobic bioremediation system performance is provided below.

Particular attention should be taken with respect to sampling groundwater, soil vapor, and soil. In reviewing a sampling plan, pay attention to the proposed sampling frequencies and methods. Some factors to look for include:

Groundwater sampling. Samples should be collected from monitoring wells located in and around the treatment area and from extraction wells (if used). Samples should not be collected from oxygen delivery wells for evaluating system performance because they would only be representative of highly localized effects of the remediation program.

Soil vapor sampling. Samples should be collected from monitoring wells located in and around the treatment area that are screened in the unsaturated zone and from soil vapor extraction wells (if used). Samples should not be collected from oxygen delivery wells for evaluating system performance because they would only be representative of highly localized effects of the remediation program.

Soil sampling. Samples should be collected from borings or using Geoprobe sampling equipment in and around the treatment area. Soil samples should consistently be collected from same contaminated sections of stratigraphic interval for comparison to earlier samples from same locations and depths.

Evaluation Criteria

The evaluation sampling described above provides evidence needed to assess the enhanced aerobic bioremediation system performance. This evidence requires examination and interpretation to confirm enhanced aerobic bioremediation system effectiveness and whether system modifications may be warranted. A discussion of these data and how system performance can be interpreted is provided below. In particular, an evaluation of performance is examined from the following two broad enhanced aerobic bioremediation system requirements:

- Oxygen delivery and distribution
- Aerobic biodegradation

Each of these is described in more detail in the following paragraphs.

Oxygen Delivery and Distribution. Performance sampling may indicate that the enhanced aerobic bioremediation system is meeting design specifications for oxygen delivery and distribution if the data show the following:

- Vadose zone air sampling suggests that there are negligible losses of supplied oxygen to the atmosphere

- Oxygen is being delivered to the subsurface at the mass delivery rate required by the design

- Dissolved oxygen levels in groundwater samples collected across the target treatment area have been elevated to concentrations of 2 mg/L or more and reduction/oxidation conditions are uniformly in the aerobic range (greater than or equal to 750 mV)

If the performance monitoring data suggest that one or more of these conditions is not met, the system may not be meeting the requirements of the design and system adjustments or modifications may need to be made. As previously discussed, the remedial system design should include contingency planning that explores performance deficiency scenarios and identifies possible solutions.

Oxygen delivery deficiencies can normally be overcome by adjusting system flow rates or upgrading equipment capacities. However, occasionally, oxygen delivery rates may be limited by the capacity of the subsurface to absorb and/or transport the delivered oxygen mass. This may occur if an infiltration system component becomes hydraulically overloaded by the infiltration rates needed to meet the design oxygen delivery objectives. Also, groundwater could become over-saturated with dissolved oxygen at injection points requiring oxygen delivery rates to be reduced to avoid off-gassing losses of oxygen to the atmosphere. In both cases, additional infiltration or injection points could readily be added to the system to expand the oxygen delivery capacity to design-specified levels.

Loss of oxygen to the unsaturated zone and ultimately the atmosphere removes this supply of oxygen available to biodegrading microorganisms. One way to limit oxygen losses without decreasing application rates is to add application points with proportionally less oxygen delivered to each location. Another approach is to alternate the supply of oxygen to various locations in the contaminated zone, allowing existing levels of oxygen to dissipate before introducing oxygen again.

Perhaps the most challenging performance problem occurs when an enhanced aerobic bioremediation system is unable to restore and maintain aerobic conditions in a portion or multiple portions of a contaminated area. Oxygen distributed from delivery points can fail to reach target contaminated areas for many reasons:

- High biological oxygen demand in the delivery point vicinity
- Elevated soil organic content
- Low permeability heterogeneous soils
- Low hydraulic gradient and groundwater flow

Possible remedies to the performance problem include adding additional oxygen delivery points, increasing oxygen delivery rates, or enhancing hydraulic gradients and groundwater flow.

Aerobic Biodegradation. Successful oxygen delivery and distribution is probably the most important performance measure for an enhanced aerobic bioremediation system. However, this is only part of the performance. The second part requires confirmation that enhanced in-situ biodegradation of the petroleum contaminants is occurring as a result of, and at rates anticipated by, the enhanced aerobic bioremediation design. Performance monitoring that suggests that an enhanced aerobic bioremediation system is operating effectively includes the following.

- Decreasing dissolved and sorbed petroleum contaminant concentrations (i.e., gradual reduction of subsurface petroleum mass consistent with design expectations).

- Production of carbon dioxide in the subsurface, as evidenced by baseline and subsequent vadose zone sampling and field analyses. Carbon dioxide production in the saturated zone may also be evaluated by sampling groundwater and analyzing the groundwater for total inorganic carbon.

- Significantly increased microbial activity in the contaminated area as suggested by comparison of baseline and subsequent microbial population plate counts.

If only one or two of these conditions exist, there may not be enough evidence to conclude that bioremediation is a significant contributor to contaminant reduction or to conclude that the enhanced aerobic bioremediation system is effective. For example, apparent contaminant reductions in dissolved and sorbed phases could occur as a result of groundwater advection and dispersion or simply because of natural fluctuations in water levels. Or, if hydraulic manipulation (engineered hydraulic gradients) of the groundwater is part of the enhanced aerobic bioremediation system, apparent contaminant reductions could result from dilution or separation of the groundwater from the contaminated soil (e.g., if the water table is depressed below the contamination). In this case, contamination levels in groundwater could rebound to near preexisting concentrations if the hydraulic controls are turned off and groundwater re-contacts the contaminated soil.

The appearance of significant levels of carbon dioxide subsequent to enhanced aerobic bioremediation system activation is a good indicator of enhanced biological activity. However, if elevated carbon dioxide levels in the unsaturated zone are unable to be detected, this does not necessarily mean that microbial activity has not been enhanced. Carbon dioxide entering the vadose zone may be diluted by pore space air exchanges with the atmosphere, operation of vapor control systems, and other means, making it difficult to distinguish small differences in concentrations.

Possibly the most direct indication that enhanced aerobic bioremediation has increased the number of hydrocarbon degrading bacteria is observation of significantly increased populations of heterotrophic bacteria in the target treatment area. While larger populations of heterotrophic bacteria may not always translate to increased levels of petroleum hydrocarbon biodegradation, the increased number of bacteria over the baseline levels would serve as a strong indicator of biodegradation. If performance sample analyses detect intermediate degradation daughter products, this may be further evidence of contaminant biodegradation that has been enhanced.

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Checklist: Can Enhanced Aerobic Bioremediation Be Used At This Site?

This checklist can help to evaluate the completeness of the corrective action plan and to identify areas that require closer scrutiny. In reviewing the corrective action plan, answer the following questions. If the answer to several questions is "no", request additional information to determine if the proposed enhanced aerobic bioremediation technology and approach will effectively accomplish the site cleanup goals within a reasonable period of time.

1. Site Factors

Yes	No	
o	o	Is the soil hydraulic conductivity greater than 10^{-7} ft/s ?
o	o	Is the soil generally free of impermeable or low permeability layers that could retain significant petroleum contaminant mass and limit the bioavailability of this mass?
o	o	Does the soil profile of the contaminanted zone contain only limited natural organic material (e.g., layers of peat or humic material)?
o	o	Is the dissolved iron concentration in the site groundwater < 10 mg/L?
o	o	Have imminent likely excessive risks to human health or the environment (if any, associated with the petroleum contamination) been eliminated?
o	o	Does the state have specific permitting requirements?

2. Enhanced Aerobic Bioremediation Design

Yes	No	
o	o	Has the mass of petroleum hydrocarbons requiring biodegradation been estimated?
o	o	Has the mass of dissolved oxygen required to biodegrade the petroleum contaminants been estimated?
o	o	Can the proposed enhanced aerobic bioremediation approach deliver the necessary oxygen mass to the treatment area within the estimated cleanup time?
o	o	Is the capacity of the enhanced aerobic bioremediation treatment system sufficient to generate and deliver oxygen at the required design rate?

o	o	Is the density and configuration of oxygen delivery points adequate to uniformly disperse dissolved oxygen through the target treatment zone, given site geology and hydrologic conditions?

3. Written Performance Monitoring Plan

Yes	**No**	
o	o	Will a comprehensive set of baseline sampling be performed prior to enhanced aerobic bioremediation system start-up?
o	o	Does the plan specifically exclude sampling from oxygen delivery wells when collecting data to evaluate enhanced aerobic bioremediation system performance?
o	o	Are monitoring wells adequately distributed between oxygen delivery locations to collect groundwater and soil vapor samples to evaluate the performance of the enhanced aerobic bioremediation system?
o	o	Does the written plan include periodically collecting soil samples from the contaminated interval(s) at locations between oxygen delivery locations?
o	o	Will the soil, soil vapor and groundwater samples be analyzed for the majority of the recommended performance monitoring parameters?
o	o	Will frequencies of performance monitoring generally correspond to those identified in Exhibit XII – 25?