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How To Evaluate Alternative Cleanup Technologies For Underground Storage Tank Sites

A Guide For Corrective Action Plan Reviewers

Chapter XIV

Enhanced Anaerobic Oxidative Bioremediation

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Enhanced Anaerobic Oxidative Bioremediation

Overview

There are two types of enhanced anaerobic bioremediation: reductive, which is an established technology mainly used to treat chlorinated compounds, and oxidative, a less common application used to treat petroleum hydrocarbons. Because underground storage tank corrective actions deal mainly with petroleum hydrocarbons, this chapter focuses on enhanced anaerobic oxidative bioremediation (EAOB). EAOB can be used to fully biodegrade a variety of contaminants in situ.

In both aerobic and anaerobic conditions, oxidation is the primary metabolic pathway by which petroleum hydrocarbons are biodegraded. A petroleum hydrocarbon is oxidized when an electron moves from the petroleum hydrocarbon, an electron donor, to another compound known as an electron acceptor. Aerobic oxidation occurs when oxygen is the electron acceptor; anaerobic oxidation occurs when compounds such as sulfate, nitrate, manganese, ferric iron, or carbon dioxide act as the electron acceptors. The availability of electron acceptors is often the limiting factor in the naturally occurring biodegradation of petroleum hydrocarbons. However, it is possible to enhance the rate of natural biodegradation by supplying additional electron acceptors to the subsurface microbial community. Aerobic bioremediation, which is discussed in this manual's Chapter XII: Enhanced Aerobic Bioremediation, occurs when a cleanup technology supplies oxygen. Enhanced anaerobic bioremediation occurs when a cleanup technology supplies an electron acceptor other than oxygen.

Researchers have found that anaerobic bioremediation can be useful for treating petroleum contamination while avoiding some of the complications, such as unintended exothermic reactions, that can arise when using aerobic bioremediation or in-situ chemical oxidation. It is advantageous to promote anaerobic bioremediation where oxygen levels are already depleted, an appropriate metabolic pathway exists for the target contaminants, and other conditions are conducive to this approach (EPA 2013). In general, aerobic conditions allow for more rapid biodegradation of reduced contaminants than anaerobic conditions. For this reason, remediation strategies often introduce oxygen or other engineered oxidants to anaerobic environments in order to convert them into aerobic environments and thereby achieve a higher rate of degradation. Although the possibility of more rapid site remediation exists with aerobic bioremediation, some disadvantages may make anaerobic bioremediation preferable:

- Numerous other subsurface mechanisms rapidly consume supplied oxygen, reducing the amount of oxygen available for biodegradation of contaminants and increasing the mass and associated costs of oxidant addition. For example, naturally occurring dissolved metals such as iron and manganese will react with some of the introduced oxygen.

- The rapid depletion of introduced oxygen can make it difficult to distribute oxygen uniformly throughout the contaminated zone, necessitating tighter spacing of oxidant injection locations and increases project costs.

- Oxygen is less soluble in water than other electron acceptors are. Therefore, the amount of electron acceptor that you can supply to microorganisms is lower when oxygen is used as the electron acceptor than when non-oxygen electron acceptors are used.

- Aerobic processes can cause biofouling, which is a build-up of microbial biomass caused by the higher growth yields associated with aerobic metabolism. Fouling leads to higher operation costs (Farhadian et al. 2008); for example, you may need to redevelop fouled wells to improve remedial performance.

Note that some researchers suggest a combined approach, in which you use both oxygen and other electron acceptors to maximize the opportunities for bioremediation (Cunningham et al. 2001). In practice, it is common to use aerobic biodegradation to reduce contaminant concentrations because of its higher degradation rates, and anaerobic biodegradation to polish residual contaminant levels to meet regulatory standards or guidelines over time.

Anaerobic bioremediation takes place in the absence of oxygen. Enhanced anaerobic oxidative bioremediation works by supplying an electron acceptor other than oxygen, such as nitrate, sulfate, or iron, to the subsurface, where anaerobic microorganisms can use the electron acceptor to degrade hydrocarbons. You can apply this approach at petroleum-contaminated sites where oxygen is depleted from the soil. Providing nutrients needed by microorganisms, called biostimulation, can enhance the conditions for anaerobic bioremediation. In limited conditions, introducing exogenous microorganisms to the subsurface, or bioaugmentation, can assist in biodegrading the target contaminants; however, bioaugmentation is generally not needed at petroleum sites because many of the microbes involved in hydrocarbon bioremediation are found in most environments.

Enhanced anaerobic oxidative bioremediation also has limitations and is not appropriate in some circumstances. For example, it is generally not practical to use EAOB to address nonaqueous-phase liquids (NAPLs) or areas with extremely high contaminant concentrations; other techniques should be used to remove as much contaminant mass as possible before employing EAOB (Cunningham et al. 2001, Hinshalwood et al. 2013, Suthersan et al. 2011). However, some studies show that anaerobic bioremediation can help address residual source material (Suthersan et al. 2011). EAOB, and other in-situ bioremediation methods that rely heavily upon contaminants coming into direct contact with oxidants or reductants present in the subsurface, are often not practical or cost-effective in low-permeability soils such as clays. In addition, because EAOB is slower than other cleanup techniques, it is not appropriate for addressing current or imminent excessive human health or environmental risks. Finally, it is often not a viable solution as a stand-alone remedy if hydraulic control is necessary to prevent contaminant migration to sensitive receptors.

Exhibit XIV-1 summarizes the general advantages and disadvantages of EAOB. Chapter X of this manual provides a general discussion of in-situ groundwater bioremediation. Some of the advantages and disadvantages of EAOB listed in Exhibit XIV-1 also apply to aerobic bioremediation methods; therefore, you should not interpret the exhibit as a comparison between aerobic and anaerobic bioremediation methods.

Enhanced Anaerobic Oxidative Bioremediation Technology Effectiveness Screening Approach

The description of EAOB in the overview provides the basic information needed to evaluate a corrective action plan (CAP) that proposes EAOB. We divide the evaluation process into the four steps described below. You can use the checklist on page XIV-73 to evaluate the completeness of the EAOB CAP and to focus attention on areas where additional information may be needed.

- Step 1: An initial screening of EAOB effectiveness allows you to quickly determine whether EAOB should be considered as a remedial approach for the site.
- Step 2: A detailed evaluation of EAOB effectiveness provides further screening criteria to confirm whether EAOB is likely to be effective. First, you need to evaluate certain site-specific data on the nature and extent of contamination, potential risk to human health or the environment, subsurface geology and hydrogeology, and other relevant site characteristics. Next, you must compare the site-specific data to the criteria provided in this document to assess whether EAOB is likely to be effective.
- Step 3: An evaluation of the EAOB system design in the CAP 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, regulatory requirements, and site-specific needs; 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 performance monitoring and contingency plans are appropriate.

Exhibit XIV-1
Enhanced Anaerobic Oxidative Bioremediation: Primary Advantages And Disadvantages

Advantages	Disadvantages
• Works with and enhances natural in-situ processes already at play (typically uses natural groundwater gradient, naturally occurring biodegradation) • Degrades the petroleum contamination in place • Can be a low-energy approach • Is generally less expensive than more aggressive remedial techniques except where NAPL or very high concentration contamination is present • Complements more aggressive technologies (e.g., groundwater extraction) and less aggressive approaches (for example, intrinsic remediation) that can be integrated into site remediation • Causes minimal disturbance to site operations and has relatively simple operation and monitoring requirements • Avoids some of the limitations of enhanced aerobic bioremediation (for example, low solubility of oxygen, aquifer clogging due to iron precipitation, biofouling) • Can be used in tandem with other remedial technologies that address small amounts of residual soil and groundwater contamination	• There is not extensive information on cost and performance over a variety of field conditions • Use of sulfate may create hydrogen sulfide • 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 injection of electron acceptor and nutrients • May or may not be fully effective on all petroleum hydrocarbons and product additives (e.g., methyl tertiary-butyl ether (MTBE) • Often must be accompanied by other technologies (e.g., product recovery) to address source areas or if hydraulic control is necessary

Initial Screening Of Enhanced Anaerobic Oxidative Bioremediation

This section allows the CAP reviewer to determine quickly if a site is not a good candidate for EAOB.[1] The main questions to ask during the initial screening are:

- Are there NAPL or high concentrations of dissolved or adsorbed-phase contaminant in the subsurface?
- Does the site pose an urgent risk to human health or the environment? For example, is vapor intrusion a concern for occupied buildings above or near the groundwater plume?

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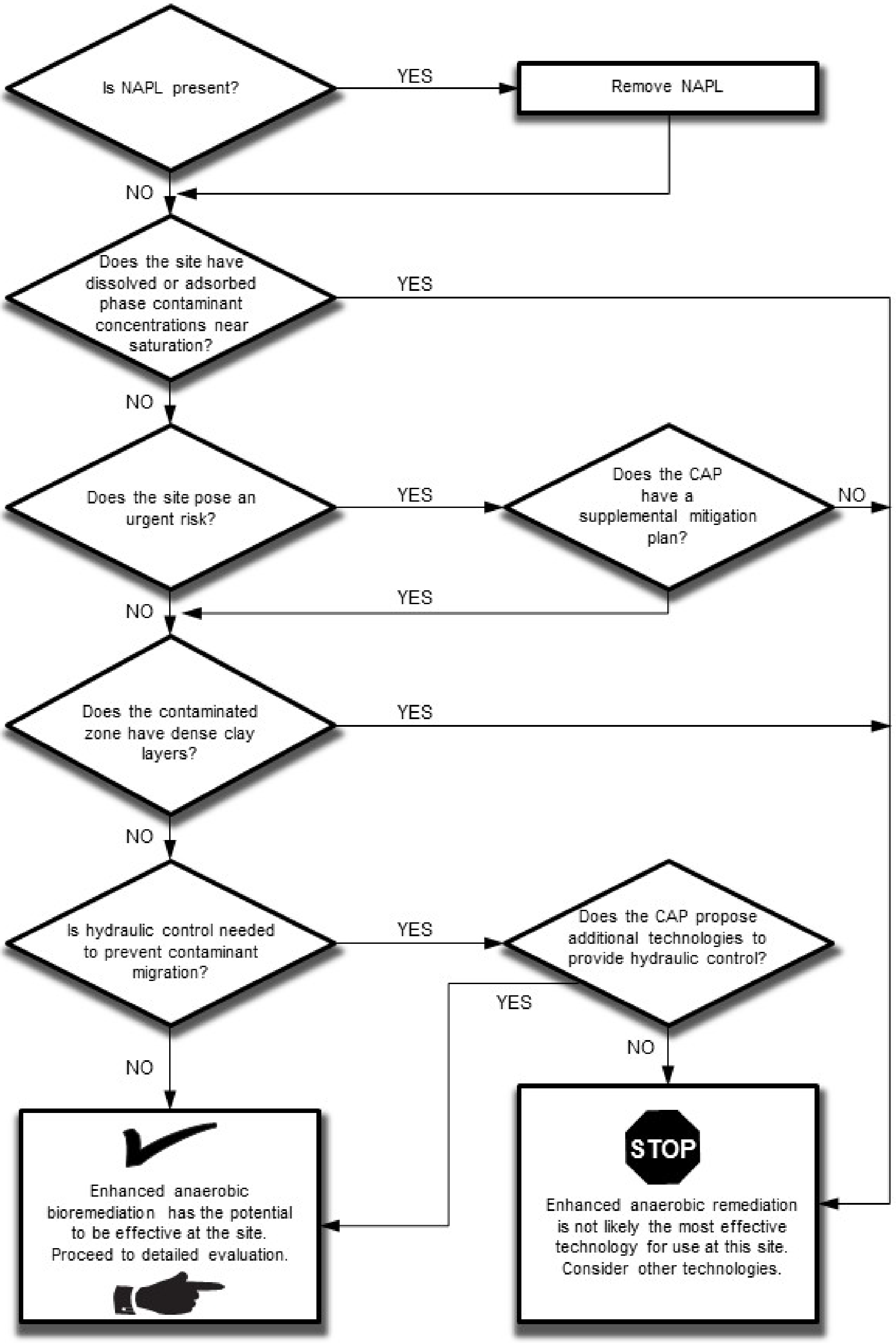
[1] This chapter focuses on anaerobic oxidative bioremediation, rather than anaerobic reductive bioremediation. See the References section for a list of information sources on anaerobic reductive bioremediation.

- Does the contaminated zone have dense clay layers?
- Is there a potential plume migration concern that cannot be addressed with EAOB?
- Do state or local regulations prohibit injection of amendments into groundwater?

If review of the CAP indicates that any of the above conditions exist, EAOB may not be a feasible or appropriate remedial solution for the site. Exhibit XIV-2 is an initial screening flowchart that helps CAP reviewers assess the potential effectiveness of EAOB. Note that the factors identified in Exhibit XIV-2 also apply to other in-situ bioremediation methods. This section offers general guidance about the applicability of EAOB, but there may be site-specific exceptions to these rules. It may be appropriate, for example, for CAPs to use EAOB to address contamination on the periphery of a plume's anaerobic zone, while employing a different technology to treat the source zone.

Exhibit XIV-2
Initial Screening For Potential Effectiveness Of Enhanced Anaerobic Oxidative Bioremediation



EAOB, by itself, will not effectively address NAPL, or extremely high dissolved or adsorbed phase contaminant concentrations at or near saturation, that will serve as an on-going source of dissolved phase contamination (Suthersan et al. 2011, Cunningham et al. 2001). If NAPL is present, then the CAP should include plans for its recovery, using a technique other than in-situ bioremediation, for EAOB to achieve cleanup goals within an acceptable timeframe.[2] Biodegradation of petroleum hydrocarbons occurs predominantly in the dissolved-phase because the compounds must be transported across the microbial cell boundary along with water, nutrients, and metabolic waste products. Therefore, in the presence of NAPL, the rate at which the NAPL dissolves into groundwater limits the rate of hydrocarbon mass destruction using EAOB. Because petroleum hydrocarbons have very low solubility unless amended with something such as a surfactant, cleaning up NAPL using bioremediation alone would likely take many years and require very large quantities of supplied electron acceptors.[3]

If the site poses an urgent risk to human health or the environment, then the CAP should include a supplemental mitigation plan. EAOB, as well as all other oxidative bioremediation methods, may not reduce contaminant concentrations quickly enough to address urgent risks. CAPs should use technologies that complement EAOB, such as soil vapor extraction (SVE) or hydraulic controls, to mitigate risks associated with petroleum contamination near basements, utilities, water supply wells, private water wells, surface water bodies, or other potential sensitive receptors.

The hydraulic conductivity of the aquifer is one of the primary factors affecting the distribution of electron acceptors and nutrients in the subsurface. To be successful, EAOB systems, like all in-situ oxidative bioremediation methods, must distribute electron acceptors throughout the treatment zone, allowing microbial populations to increase, come into contact with, and metabolize the petroleum contaminants. If the site’s contaminated zone contains dense and impermeable layers, such as clay, it may be difficult or impossible to distribute the electron acceptors throughout the contaminated zone without extensive costs. Fine-grained media (clays and silts) have lower intrinsic permeability than coarse-grained media such as sands and gravels. Bioremediation is generally more effective in permeable aquifer media. In general, an aquifer medium of lower permeability will require more time and higher cost to clean up than a more permeable medium.

Detailed Evaluation Of Enhanced Anaerobic Oxidative Bioremediation

After the reviewer of the CAP has completed the initial screening and determined that EAOB may be effective for the site, the reviewer should evaluate the CAP further to confirm that EAOB could be effective. The initial screening focused on NAPL, urgent risks, hydraulic control needs, and hydraulic conductivity; the detailed evaluation should consider a broader range of site and constituent characteristics, which we list in Exhibit XIV-3 and present in the Exhibit XIV-4 flowchart. These site and constituent characteristics compose the corrective action plan’s conceptual site model. The detailed

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[2] For NAPL recovery approaches, see *How To Effectively Recover Free Product At Leaking Underground Storage Tank Sites: A Guide For State Regulators*, EPA 510-R-96-001, September 1996.

[3] Note that some recent research supports the use of enhanced anaerobic reductive bioremediation to treat chlorinated dense nonaqueous phase liquid (DNAPL) source zones (Interstate Technology and Regulatory Council 2008).

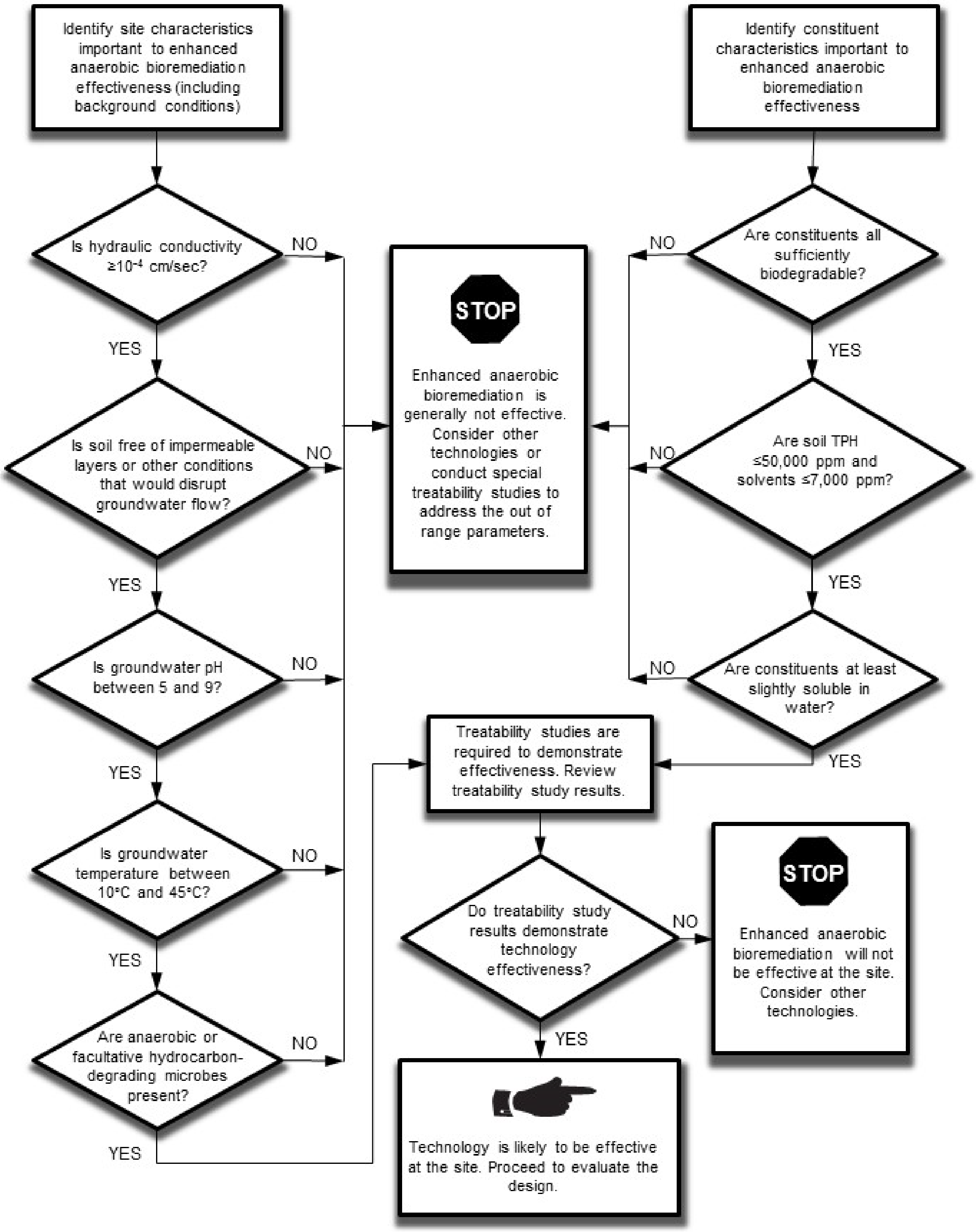
evaluation should include a detailed biogeochemical evaluation of background conditions. Note that many of the items in Exhibits XIV-3 and XIV-4 also apply to other in-situ bioremediation methods in addition to EAOB.

Exhibit XIV-3
Key Parameters Used To Evaluate The Effectiveness Of Enhanced Anaerobic Oxidative Bioremediation

Site Characteristics	Constituent Characteristics*
• Proximity of site to receptors • Hydraulic conductivity and soil permeability • Hydraulic gradient and elevation • Soil structure and stratification • Oxidation-reduction potential • Groundwater mineral content • Groundwater pH • Groundwater temperature • Microbes present: types, numbers, and distribution • Terminal electron acceptors • Nutrient concentrations • Heterogeneity of geology	• Biodegradability • Location of contamination • Chemical structure • Concentration and toxicity • Solubility • Soil sorption • Toxicity of degradation products • Heterogeneity of contamination

* NOTE: constituent characteristics apply to petroleum as well as other constituents including inorganics, nutrients, etc.

Exhibit XIV-4
Detailed Screening For Potential Effectiveness Of Enhanced Anaerobic Oxidative Bioremediation



Site Characteristics Affecting Enhanced Anaerobic Oxidative Bioremediation

Site characteristics that influence the potential effectiveness of EAOB are described below. While we outline important issues that require consideration in the implementation of a successful EAOB strategy, please note that many of these issues are not specific to anaerobic metabolism and are equally important when considering any in-situ bioremediation method.

Hydraulic Conductivity And Soil Permeability

Hydraulic conductivity (K), which is a measure of water's ability to move through the aquifer medium, is one of the most important factors in determining the effectiveness of subsurface delivery systems. This characteristic is one of the main factors controlling the rate and the distribution of electron acceptors and nutrients delivered to the microorganisms in the aquifer as well as the location and extent of the contamination. An aquifer's hydraulic conductivity depends in part on the size of the particles that make up the aquifer. The smallest particles are clay, followed by silt, sand, and gravel. In general, fine-grained soils composed of clays or silts offer greater resistance to water flow. Soils that are highly fractured, however, may have sufficient permeability to use in-situ bioremediation regardless of particle size. You should be aware that preferential flow through the fractures might cause injected chemicals to travel mainly through fractures, as opposed to penetrating the entire soil matrix.

For aquifers with hydraulic conductivity greater than 10^{-4} cm/sec, in-situ groundwater bioremediation may be a technically effective and cost-effective method. For sites with lower hydraulic conductivities (e.g., 10^{-4} to 10^{-6} cm/sec), the technology also could be technically effective, but may not be cost-effective, for example due to the need for more wells; at these sites, in-situ groundwater bioremediation must be carefully evaluated, designed, and controlled.

You can estimate hydraulic conductivity in the field by slug tests, aquifer pumping tests, and vertical hydraulic profiling. Hydraulic conductivities can differ by orders of magnitude within an aquifer, so a single measurement of K may not adequately describe an aquifer. Slug tests are relatively affordable and can be completed at several wells to help understand the distribution of K across a site, but it can be difficult to evaluate K if significant differences are present between the gravel pack and aquifer materials. Conversely, aquifer-pumping tests tend to average K values because of their greater radius of influence and may provide less detail regarding a specific location or specific depth interval. Vertical hydraulic profiling provides high-resolution K data but cannot provide specific information regarding the connectivity between higher conductivity zones in various profiles, as an aquifer-pumping test could. In addition, vertical profiling provides an index of relative K at very small scale, versus measured K at larger scale, which is useful for understanding site heterogeneity. Often, a combination of aquifer test methods is best for designing an in-situ bioremediation system. The CAP must carefully design these tests to ensure that contaminants are not inadvertently forced to spread further in the aquifer and that a large volume of contaminated groundwater is not generated, which then requires expensive treatment or disposal.

Intrinsic permeability, which is a measure of the ability of soils to transmit fluids, is sometimes reported instead of hydraulic conductivity. If intrinsic permeability is given, one can calculate the hydraulic conductivity using the following equation:

$$K = \frac{k(\rho g)}{\mu}$$

where:

K = hydraulic conductivity (cm/sec)
k = intrinsic permeability (cm^2)
μ = water viscosity (g/cm-sec)
ρ = water density (g/cm^3)
g = acceleration due to gravity (cm/sec^2)

At 20°C, $(\rho g/\mu) = 9.8 \times 10^4$ $(cm\text{-}sec)^{-1}$. To convert k from cm^2 to darcy, multiply by 10^8.

Groundwater Flow Direction And Velocity

The direction of groundwater flow is a key factor driving contaminant transport. Groundwater flows from high to low hydraulic head. These hydraulic heads are often represented on potentiometric surface maps, typically referred to as water or groundwater level maps. Groundwater flow velocity, or seepage velocity, is a measure of the groundwater flow rate through the aquifer pore space. Groundwater flow velocity is an important parameter for selecting injection well placement and amendment quantities and type. For example, sites with high groundwater flow velocities may require amendment to be added more frequently than sites with low groundwater flow velocities.

High groundwater flow velocities can sometimes be advantageously incorporated into the amendment delivery design. For example, injection wells can be placed upgradient of inaccessible areas, such as under buildings, to deliver amendments. This allows the natural and additional induced flow to transport the amendments to the target treatment areas. However, if the injection rate is too high, the induced gradient may result in an undesirable increase in the rate of migration of the contaminant plume and it may migrate in an undesirable or unintended direction.

Preferential pathways exist in many geologic settings because of the heterogeneous distribution of more transmissive zones caused by coarser-grained sediments or highly fractured bedrock. Groundwater flow velocity through these zones will be higher than the average groundwater flow at the site. In addition, injection pressures created by the application of amendments can exaggerate the groundwater flow velocity and direction through preferential pathways. The increased groundwater flow velocities can cause amendments to travel beyond the target application area or reach the ground surface.

EAOB technologies ultimately rely on groundwater advection and dispersion, or flow, to distribute amendments to the subsurface. Distribution of amendments 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. As the hydraulic gradient increases, the groundwater velocity increases proportionately. Soils with higher permeability have higher groundwater velocity. For purposes of evaluating the feasibility of using an EAOB technology, it is important to note that the principal direction of groundwater flow and, therefore, amendment transport is along the line of maximum hydraulic gradient.

In some situations, hydraulic gradients can be enhanced to help increase groundwater flow and amendment delivery rates through the contaminated zone. One common approach to create an artificial gradient is by removing groundwater downgradient of the source area, mixing in amendments, and reintroducing the groundwater upgradient of the contaminated area. 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 amendment, flow across the contaminated zone.

Although sites with high groundwater flow rates may have greater distribution of amendments, there are several potential drawbacks in high groundwater flow systems that must be considered during the evaluation of anaerobic bioremediation. If the groundwater flow rate is too high, injected amendments may become diluted within the desired treatment zone or flow out of the desired treatment zone too quickly, thereby reducing efficiency of injections and remedial effectiveness. In extreme cases, a groundwater recirculation system may be needed to ensure that the added amendments remain within the desired treatment zone. This type of a system may also provide the hydraulic control that is often needed in high groundwater flow systems.

Depth To Groundwater

Another important consideration is the elevation of the groundwater table and depth to groundwater at the site. On sites where the water table is shallow (generally 5 feet or less below grade surface) it may be very difficult to inject amendments without them emerging at the surface, called “daylighting.” Therefore, the hydraulic conductivity testing discussed previously should be used to determine the maximum realistic injection rate that is achievable without daylighting during the evaluation phase of the project. This determination may be one of the greatest factors influencing the cost of implementing an amendment injection program, because it directly translates to field labor costs and the duration of injection events. In practice, if the injection rate cannot exceed 0.25 to 0.5 gallons per minute due to either the presence of a shallow water table or a low hydraulic conductivity, any remedy requiring injection of amendments may not be cost-effective in comparison to other potentially applicable remedies.

Soil Structure And Stratification

Understanding the geological setting and its heterogeneity is critical to developing a successful CAP. Often, soils in a target treatment area are not uniformly permeable, but rather have large-scale or small-scale variations in permeability. In addition, there may be anthropogenic factors greatly influencing subsurface transport, such as utility bedding materials, which create preferential migration pathways, or construction footers and grade beams, which can prevent flow. Land created by historic landfilling has substantial, and often unpredictable, subsurface heterogeneity. Subsurface heterogeneity plays a very important role in EAOB technologies because electron acceptors and other amendments introduced to the subsurface are distributed preferentially along higher permeability layers in the saturated subsurface. For example, in a heterogeneous soil comprised of sand, silt, and clay layers, amendments may travel preferentially into the sand layer, with less effective distribution in the silt and clay layers. This preferential distribution may be acceptable if the contaminants of concern followed the same preferential pathway. However, this may not always be the case, so care must be taken in the design of injection systems. For the amendment to be completely effective, it needs to permeate the entire affected subsurface area. Downgradient pumping can be used to help spread the amendment, but it is subject to preferential flow patterns. Performance monitoring with geophysical techniques, such as cross-borehole radar and electrical resistivity tomography, have been used to identify areas that may be bypassed by amendments due to preferential flow, thereby requiring special attention.[4]

Diffusion, which is generally a slower transport mechanism than advection and dispersion, may become more important than advection and dispersion in the distribution of amendments to microorganisms in the silt and clay layers. If the silt and clay layers are thick relative to the sand horizon, and they contain significant petroleum hydrocarbon mass, EAOB technologies may not be efficient or effective. In this case, the dissolved petroleum hydrocarbon mass will appear to shrink as the most permeable zone (sand) will have undergone significant EAOB treatment. However, the petroleum mass in the silt and clay horizons may not biodegrade, and can subsequently diffuse into the sand zone over time, 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 EAOB 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 EAOB is likely to be effective and will achieve cleanup objectives. Investigative approaches such as the use of membrane interface probes (MIPs) or laser-induced fluorescence sensors (LIFs) are often useful to assess the distribution of petroleum contaminants within varying soil types. An MIP is a field-screening tool for detecting volatile organic compounds in subsurface soil and groundwater; MIPs use heat to volatilize and mobilize contaminants for sampling at the surface. LIF sensors are field-screening tools for detecting petroleum NAPLs in the subsurface; the sensors emit ultraviolet light and detect the resulting fluorescence emitted by polycyclic aromatic hydrocarbons present in the petroleum.

If specific soil horizons containing hydrocarbon mass are not expected to be effectively treated using EAOB, then EAOB may not be viable for the site. For example, if 50 percent of the contaminant mass is contained and isolated in low-permeability soil horizons and the site cleanup goal is a 95 percent reduction in petroleum contaminant concentrations, then it is reasonable to conclude that the goal cannot be achieved using EAOB, or any other in-situ bioremediation method. However, in such circumstances, combining EAOB with other technologies that enhance the permeability of low permeability horizons in the contaminated zone, such as soil fracturing, could be considered. Soil fracturing could allow electron acceptors and other microbial nutrients to be effectively delivered through the engineered fractures in low-permeability soil.[5] However, observe caution when considering

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[4] EPA CLU-IN. Dense Nonaqueous Phase Liquids (DNAPLs): Treatment Technologies. http://www.clu-in.org/contaminantfocus/default.focus/sec/Dense_Nonaqueous_Phase_Liquids_%28DNAPLs%29/cat/Treatment_Technologies/p/1

[5] For more information, see: EPA. In Situ Remediation Technology Status Report: Hydraulic and Pneumatic Fracturing. https://clu-in.org/download/remed/fractur.pdf

this option because the same fractures produced to enhance permeability for amendment delivery could also be a potential preferential flow path for contaminant plume migration. In addition, soil fracturing could result in poor distribution of the added electron acceptors and nutrients, if the added materials flow preferentially through the fractures, rather than through the low-permeability soil.

The intergranular structure and stratification of aquifer media can be determined by reviewing soil logs from wells or borings and by examining geologic cross-sections. It will be necessary to verify that soil types have been properly identified, that visual observations of soil structure have been documented, and that boring logs are of sufficient detail to define soil stratification. Stratified soils may require special design consideration (such as, special injection well(s)) to ensure that these less-permeable strata are adequately handled.

Fluctuations in the groundwater table should also be determined. Significant seasonal or daily tidal or precipitation-related fluctuations will submerge some of the soil in the unsaturated zone, which should be considered during design of the system.

Porosity

Porosity, or total porosity, is a measure of the void space in an aquifer. Specifically, total porosity is the volume of the void space divided by the volume of aquifer matrix. Total porosity in bedrock aquifers is described as primary and secondary. Primary porosity is the percentage of the voids in the rock at the time of formation, and secondary porosity refers to the void space from fractures and dissolution. Primary and secondary porosity are important for estimating how much contaminant mass may be present and can be useful for estimating amendment quantities. More important however, is effective porosity, which is a measure of the connected aquifer void space within the aquifer. Effective porosity is lower than total porosity in most geological settings. When effective porosity is low, it may be difficult for the amendment delivery systems to treat the target area because of the poor connections between the aquifer void space and fractures. A value for effective porosity is useful to determine the following:

- The radius of influence of an injection well
- The total number of injection wells
- Whether multiple screened intervals are required for the injection wells
- The volumes of reagents needed for injection

You can estimate effective porosity in the field by measuring groundwater flow velocity with a tracer test.

Federal Remediation Technologies Roundtable. 4.6 Fracturing (In Situ Soil Remediation Technology). http://www.frtr.gov/matrix2/section4/4-5.html
EPA CLU-IN. Environmental Fracturing. https://clu-in.org/techfocus/default.focus/sec/Environmental_Fracturing/cat/Overview/

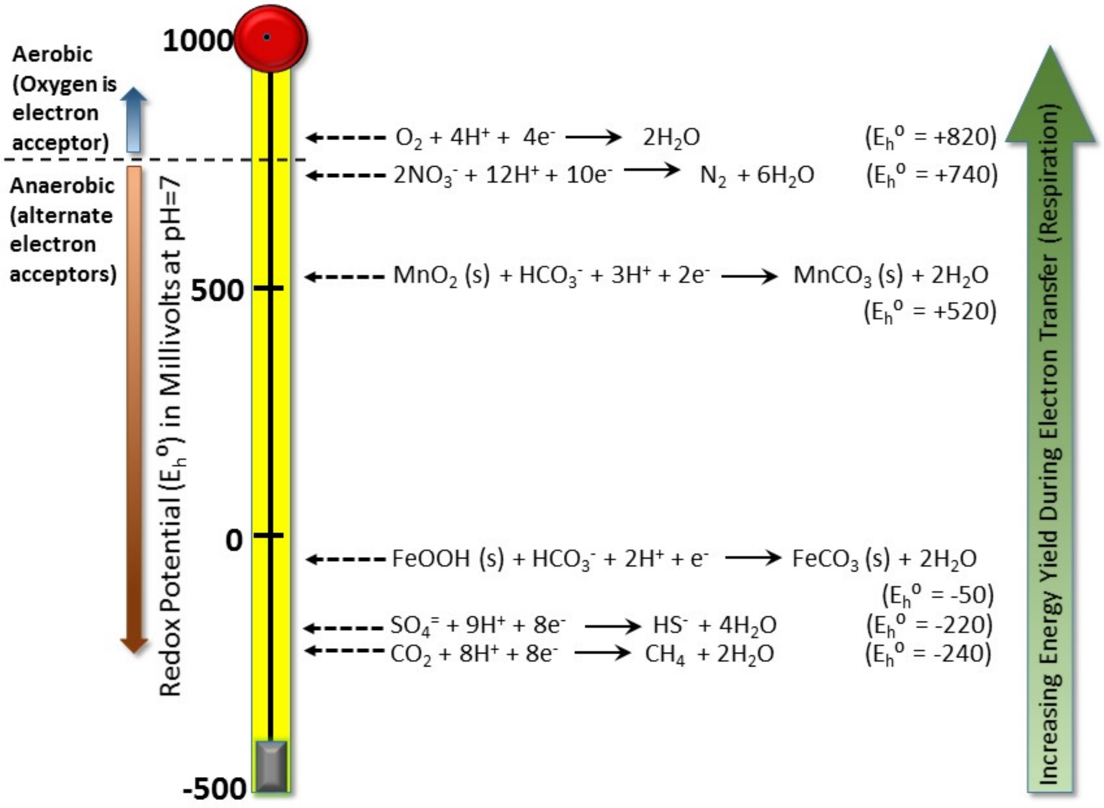
Oxidation-Reduction Potential

An aqueous solution's oxidation-reduction, or redox, potential is a measure of its tendency to either release or accept electrons. Oxidizing systems tend to accept electrons; reducing systems tend to release electrons. Excessive organic loading of an aquifer from a fuel release will result in biological activity that will readily consume oxygen and drive a system to reducing conditions. The reducing environment, in turn, may cause certain metals (for example, arsenic) to become mobile and create secondary groundwater impacts. Therefore, it is important to delineate the contaminant plume and understand the effects of the contaminants on site geochemistry. Redox potential can be a difficult parameter to measure accurately in the field. Down-hole probes or low-flow pumps with flow-through cells are typically the most accurate methods of measuring redox potential.

You measure redox potential in millivolts (mV). Many biological processes operate only within a prescribed range of redox conditions. The lower the redox potential, the more reducing and anaerobic the environment. As depicted in Exhibit XIV-5, as the redox potential of the groundwater decreases, the predominant electron acceptor changes through the following sequence: oxygen, nitrate, manganese, iron, sulfate, and carbonate.

A significant concern at many oxidation sites is that oxidation of certain metals can cause a change in valence state to a more toxic form of the metal in certain subsurface conditions, such as oxidation of trivalent chromium to hexavalent chromium. This undesirable effect can occur with both aerobic and anaerobic oxidation.

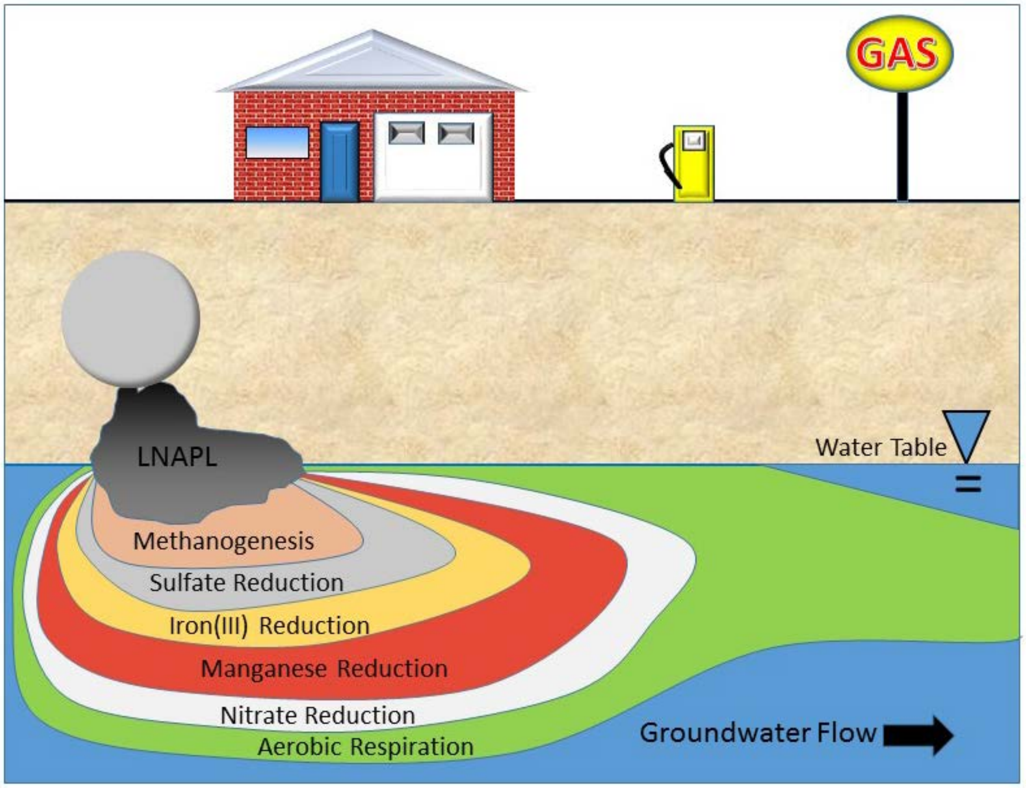
Exhibit XIV-5
Redox Potentials For Various Electron Acceptors



Terminal Electron Acceptors

Terminal electron acceptors are the compounds used by organisms for respiration via an electron transfer chain. Aerobic organisms use oxygen as the terminal electron acceptor; anaerobic organisms use a variety of other terminal electron acceptors. Organisms that can use either oxygen or an alternative electron acceptor are facultative. In the absence of oxygen, the overall microbial community will sequentially use alternative electron acceptors based on their respective redox potentials. In order of preference, microbes will use oxygen, followed by nitrate, manganese(IV) oxide, iron(III) hydroxides, sulfate, and finally carbon dioxide. Exhibit XIV-6 presents an idealized and simplified schematic of the reducing zones that you would expect at a petroleum spill.

Exhibit XIV-6
Conceptualization Of Electron Acceptor Zones In The Subsurface Downgradient Of A Petroleum Release



Measuring the concentration and distribution of electron acceptors in the subsurface prior to enhancement will help determine how much additional electron acceptor needs to be added to achieve complete contaminant biodegradation, as well as the optimal locations of these amendments.

Groundwater Geochemistry

Excessive calcium, magnesium, or iron in groundwater can react with phosphate, which is typically supplied in EAOB as a nutrient in the form of tripolyphosphate. The products of these reactions can adversely affect the operation of an in-situ bioremediation system. When calcium, magnesium, or iron reacts with phosphate, crystalline precipitate, or scale, is formed. Scale can constrict flow channels and can damage equipment such as injection wells. In addition, the precipitation of calcium or magnesium phosphates ties up phosphorus compounds, making them unavailable to microorganisms for use as nutrients. You can minimize these negative effects by using tripolyphosphates in a mole ratio of greater than 1:1 tripolyphosphates to total minerals, such as magnesium and calcium. At these concentrations, the tripolyphosphate acts as a sequestering agent to keep the magnesium and calcium in solution, as it will help prevent the metal ions from precipitating and forming scale.

Other parameters that could be good indicators of potential groundwater scaling are hardness, alkalinity, and pH. Very hard water (greater than 400-500 mg/L carbonate hardness) tends to promote scaling. The potential adverse effects caused by excessive mineral content, such as calcium, magnesium, iron, or total carbonates, in the groundwater warrant careful attention during site characterization activities.

Groundwater pH

Extreme pH values, less than 5 or greater than 10, are generally unfavorable for microbial activity. Typically, optimal microbial activity occurs under neutral pH conditions in the range of 6–8. However, biological treatment at sites on the margins or just outside the optimal pH ranges should not be rejected until further site biological screening, such as bench testing, is completed. The optimal pH is site-specific. For example, robust microbial activity has been observed in natural systems at lower pH conditions outside of the neutral pH range, such as pH 4.5 to 5, as stated in Chapter X of this manual. Because indigenous microorganisms have adapted to the natural conditions where they live, pH adjustment, even toward neutral, can inhibit microbial activity. If man-made conditions, such as releases of petroleum, have altered the pH outside the range observed in nearby background samples, pH adjustment may be needed. If the pH of the groundwater is too low, or too acidic, lime (calcium hydroxide) or sodium hydroxide can be added to increase the pH. If the pH is too high, or too alkaline, then a suitable acid, such as hydrochloric acid, can be added to reduce the pH. Changes to pH should be closely monitored because rapid changes of more than one or two units can inhibit microbial activity and may require an extended acclimation period before the microbes resume their activity. However, you should be wary of changing environmental pH as it can lead to adverse mineral precipitation and clogging.

EAOB can cause the pH of the subsurface to increase or decrease. The CAP should consider this possibility and, if necessary, include plans to measure the buffering capacity of the aquifer prior to remediation. There are two laboratory approaches to measure buffering capacity. If an aquifer matrix is rich in limestone and a high natural pH buffering capacity is anticipated, a laboratory acid titration test can be completed on site soil and groundwater samples to determine the level of acid equivalents that will reduce pH to levels outside optimal limits. This approach is referred to as alkalinity testing. The other approach is acidity testing, which is appropriate if a site is anticipated to have a low buffering capacity, or is in active remediation with low pH conditions. Acidity testing consists of adding a base (such as sodium bicarbonate or sodium hydroxide) to site soil and groundwater in a laboratory setting to determine the equivalents of base needed to overcome aquifer acidity and maintain a near-neutral pH. Alkalinity and acidity tests also provide insight on how the potential buffering requirements of the aquifer may affect the feasibility of the planned bioremediation.

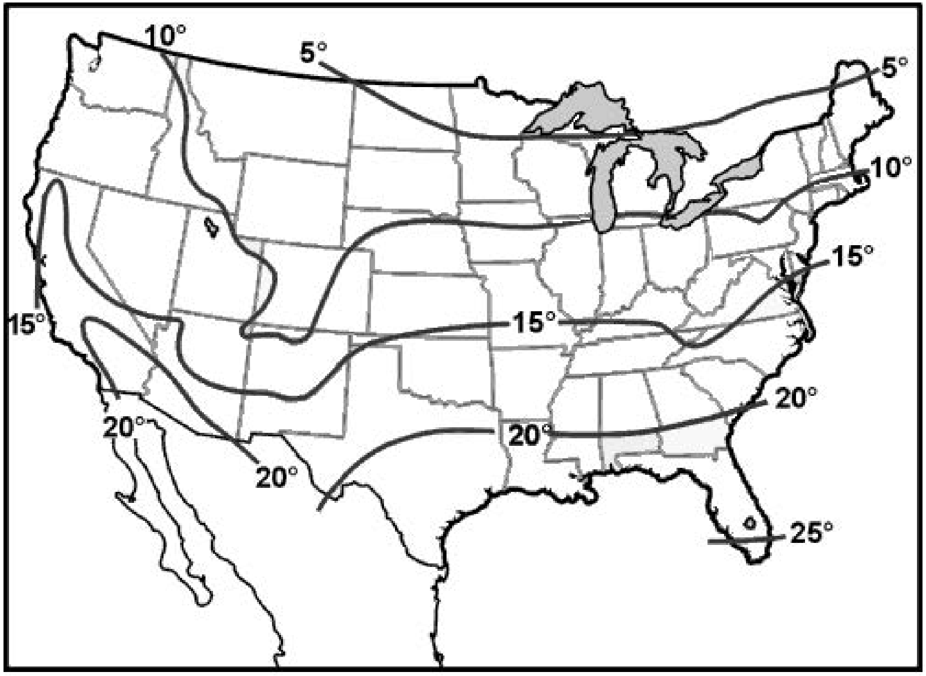
If EAOB is being considered in conjunction with other remedial approaches, for example as a polish to treat low-level residual wastes following oxidation, pH concerns can be magnified because many other remedial approaches may necessitate a raising or lowering of pH to be effective. It may be necessary to adjust pH in-situ before starting the EAOB phase of cleanup under this type of scenario. This additional round of pH adjustment may reduce the cost-effectiveness of using EAOB under this type of scenario and will negatively affect the microorganisms.

Besides the direct effect that pH has on microorganisms, the pH can also have other effects that could negate the efficacy of bioremediation. For example, a decrease in pH can solubilize toxic metals that were previously insoluble and create secondary environmental impacts. If an aquifer is known or suspected to contain metals that may solubilize if pH is lowered, the practitioner may choose to add a buffering agent to prevent pH from decreasing to a range that may solubilize metals. In addition, the CAP should determine whether any utilities within the treatment area could be adversely impacted by the added amendments. For example, if an amendment affects pH, then the CAP should determine whether any utility components (such as piping or seals) within the treatment area are incompatible with the expected pH.

Groundwater Temperature

Bacterial growth rate is a function of temperature. Subsurface microbial activity in non-extreme environments decreases significantly at temperatures below 10°C and essentially ceases below 5°C. Therefore, bioremediation in northern climates, especially during winter months, will be slower and may require additional design considerations (EPA 2013). The activity of most bacterial species important to petroleum hydrocarbon biodegradation also diminishes at temperatures greater than 45°C. Within the range of 10°C to 45°C, the rate of microbial activity typically doubles for every 10°C rise in temperature. In most cases, for in-situ groundwater bioremediation, the bacteria living in an aquifer system are likely to experience relatively stable temperatures with only slight seasonal variations. In most areas of the United States, the average groundwater temperature is about 10°C to 20°C, but groundwater temperatures may be somewhat lower or higher in the extreme northern and southern states, respectively, as shown in Exhibit XIV-7.

Exhibit XIV-7
Average Temperature Of Shallow Groundwater



Source: EPA 2013

Microbial Presence

Soil normally contains large numbers of diverse microorganisms, including bacteria, algae, fungi, protozoa, and actinomycetes. Of these organisms, bacteria are the most numerous and biochemically active group, particularly at low oxygen levels, and they contribute significantly to in-situ groundwater bioremediation. At a contaminated site, the natural microbial ecology undergoes a selection process. First, there is an acclimation period, when surviving microbes adjust to their new environment. Some of these microbes adjust their metabolism such that the contaminant becomes a new source of food. Second, those organisms that adapt most quickly tend to grow fastest and can use up nutrients that other microbes would need. Third, as the environmental conditions continue to change and the nature of the food supply changes, the microbial populations continue to change as well.

To determine the presence and population density of naturally occurring bacteria that will contribute to degradation of petroleum constituents, laboratory analysis of soil samples from the site should be completed. These analyses, at a minimum, should include an enumeration of hydrocarbon-degrading bacteria, such as high G+C Gram-positive bacteria, *Nocardioides*, *Arthrobacter*, and total heterotrophic bacteria that use organic compounds, such as simple sugars and hydrocarbons, as an energy source. When quantifying the microbial populations at a site to assess the applicability of EAOB, anaerobic or facultative hydrocarbon-degraders are the most relevant species to enumerate. Quantifying heterotrophic bacteria in general, both aerobic and anaerobic, is less relevant to the applicability of EAOB, because heterotrophic bacteria are not necessarily degrading site target hydrocarbons. However, if a site lacks all types of heterotrophic bacteria, then that may indicate that site conditions are not favorable for bioremediation.

Molecular biological tools are becoming more widely available and cost effective for applications in support of site characterization, remediation, and monitoring to determine microbial populations within aquifers. Quantitative Polymerase Chain Reaction (qPCR) is the most commonly used method to determine microbial populations. qPCR can be used to quantify the abundance of a desired microbial population capable of biodegrading the contaminants found at the site. Other methods include micro assays and fluorescence in situ hybridization (FISH) (ITRC 2013). These commercially-available methods are much more accurate and reliable than plate counts, and should be used instead of plate counts whenever possible.

Although conditions in a contaminated aquifer may not be favorable for bacteria to thrive in the subsurface, the presence of a specific target bacteria, such as a known petroleum hydrocarbon degrader, in an aquifer may be a strong indication that bioremediation is feasible. However, the absence of a target microbial population does not preclude application of bioremediation at a site. In many instances, suitable microorganisms may be present at a site, but at population densities that are too low to detect by non-molecular methods. In these instances, populations may be increased and strengthened by biostimulation or, in limited cases, bioaugmentation. The cost of amendments and their delivery to the target treatment zones are often the highest portion of total project costs; therefore, sites requiring significant bioaugmentation must be screened more carefully than sites where natural microbial populations of interest are already present and thriving.

Bioaugmentation

Some CAPs propose the addition of microorganisms to the aquifer environment when target population sizes are low, known as bioaugmentation. However, you generally do not need bioaugmentation at petroleum sites because many of the bacteria involved in hydrocarbon bioremediation are found in most environments. Research has shown that most in-situ bioremediation projects have been successfully completed without microbial augmentation, as seen in Chapter X of this manual, as well as EPA 2013, p. 18, and El Fantroussi and Agathos 2005, p. 269. Experience with microbial augmentation indicates that it varies in effectiveness. Except in coarse-grained, highly permeable material, microbes tend not to move very far past the point of injection; therefore, their effectiveness following augmentation injections is limited in extent. In general, microbial augmentation does not adversely affect bioremediation, but it could be an unnecessary cost.

Constituent Characteristics Affecting Enhanced Anaerobic Oxidative Bioremediation

It is important to evaluate the potential impacts of site contaminants on the performance of the proposed EAOB 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.

Biodegradability

Petroleum products are complex mixtures of hundreds or thousands of hydrocarbon chemical constituents, other chemical constituents, and additives. Each of these constituents has a different chemical structure that determines, in part, its relative biodegradability. For example, bioremediation does not degrade heavy metals, which may be present in waste oils, but they may be transformed into more or less soluble forms. The biodegradability of organic constituents depends on their chemical structures and physical and chemical properties, such as water solubility and water/octanol partition coefficient. Highly soluble organic compounds with low molecular weights, such as toluene, ethylbenzene, and xylenes, tend to degrade more rapidly than slightly soluble compounds with high molecular weights. Ethanol, a common fuel additive, is highly biodegradable; its presence may delay the biodegradation of less preferred substrates such as petroleum hydrocarbons; see page XIV-29 for information about *Special Considerations For MTBE And Other Fuel Oxygenates*. The low water solubilities of the more complex compounds render them less bioavailable to petroleum-degrading organisms because biodegradation occurs in the aqueous phase. Consequently, the larger, more complex chemical compounds may be slow to degrade or may even be recalcitrant to anaerobic biological degradation. Some studies have demonstrated that surfactants can enhance the biodegradation of hydrocarbons (Mulligan et al. 2001). Exhibit XIV-8 describes the relative biodegradability of various petroleum products. Although, in general, petroleum contaminants with higher solubilities are more readily biodegradable, there are some exceptions. For example, although benzene is the most soluble of the BTEX compounds, made up of benzene, toluene, ethylbenzene, and xylenes, as seen in Exhibit XIV-10, it is the most recalcitrant to biodegradation (Johnson et al. 2003).

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. Generally, 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.

After a petroleum release occurs, the petroleum becomes increasingly weathered over time due to physical, chemical, and biological processes. Researchers have observed that more highly weathered petroleum is often less biodegradable than fresher petroleum, perhaps because the more biodegradable components have already degraded, leaving the more recalcitrant components, or because the petroleum molecules become more highly sorbed over time (Maletic et al. 2013).

The most common contaminants at leaking underground storage tank sites are gasoline and diesel. Within the gasoline category, benzene will biodegrade anaerobically after other preferentially degraded hydrocarbons have been removed (Cunningham et al. 2001). Naphthalene, phenol, cresol, and benzoic acids are aromatic hydrocarbons that are most readily degraded anaerobically (Coates et al. 1996, Meckenstock 2000, Suflita 1991).

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 depend on constituent, site, and implementation specific conditions. For example, the mixture and concentrations of the different petroleum constituents in the soil and groundwater 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.

Evaluation of the chemical structure of the constituents proposed for treatment by EAOB at the site will allow you to determine which constituents will be the most difficult to degrade. You should verify that remedial time estimates, treatability studies, and operation and monitoring plans are based on the constituents that will take the longest time to degrade, unless another remedial technology is being proposed to address those compounds. Note that it is not the 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 EAOB design and cleanup. As remediation progresses, the mix of petroleum products remaining should periodically be compared to the site's proposed cleanup levels to determine whether the remedial approach needs to be enhanced to address the remaining target compounds.

Exhibit XIV-8
Composition And Relative Biodegradability Of Petroleum Products

Category	Components	Relative Biodegradability By Anaerobic Oxidation
Additives	Ethanol, methanol	HIGHER ↕ LOWER
Gasoline – Normal and branched hydrocarbons between 6 and 10 carbons in length	toluene, xylenes	
	ethylbenzene[6]	
	benzene	
	other gasoline-range organics[7]	
Kerosene, Diesel	• Hydrocarbons primarily between 11 to 12 carbons long, 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 • 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	

Location Of Contamination

The location, distribution, and disposition of petroleum contamination in the subsurface can significantly influence the likelihood of success for bioremediation. Spilled petroleum products may be partitioned into one or more phases and zones in the subsurface including:

- Shallow subsurface
    - Unsaturated soil pore space (vapor phase)

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[6] EPA 2013 (Section 2.2.1)

[7] Suthersan et al. 2011

    - Unsaturated soils (sorbed phase)

- Deeper subsurface
    - Residual product smeared onto soil above and below the water table (liquid phase)
    - Free mobile product (liquid phase)
    - Saturated soil (sorbed phase)
    - Dissolved in groundwater (aqueous phase)

Understanding how the petroleum contaminant mass is distributed in the subsurface can be important to evaluating the applicability of EAOB. EAOB would be most effective for dissolved contaminants and contamination adsorbed onto higher permeability sediments, such as sands and gravels. However, if most of the contamination is in the unsaturated zone, trapped in lower permeability sediments, or outside the flow path for nutrients and electron acceptors, EAOB would have reduced impact or no impact.

A clear understanding of the contaminant distribution and contaminant phases is critical for the proper design of any remediation system. The contaminant mass distribution is a primary variable used to calculate the quantity of amendment and identify the appropriate delivery method at bioremediation sites. The lack of adequate characterization is one of the main reasons for poor remedial performance.

Source Zones

Depending on the characteristics and amount of contaminant present, contamination may be completely dissolved in the groundwater or exist as nonaqueous-phase liquid (NAPL), which is typically found within areas considered source areas. NAPL can exist as either free product or residual NAPL. The source area may contain significant contaminant mass relative to the entire contaminated area. NAPL can co-exist with water in the pore space of an aquifer. Light non-aqueous phase liquids, or LNAPLs, tend to exist in the upper portion of the aquifer, while dense non-aqueous phase liquids, or DNAPLs, tend to sink through the aquifer until they reach an impermeable formation. Most petroleum contamination is lighter than water, so it will form LNAPL rather than DNAPL. The presence of LNAPL is more readily apparent than DNAPL by direct observation of floating product in a well, oil sheen on water during sampling, and coatings on sampling equipment.[8] See the Initial Screening section of this chapter for more information about source zones.

When NAPLs of either kind move through soils and aquifer materials, they leave behind immobile, discontinuous droplets referred to as residual NAPL, which is difficult to find and recover. A pool of LNAPL at the water table will create an area of residual NAPL called a smear zone as the water table fluctuates over time. The smear zone "…can present a challenge to effective reagent delivery…" (Suthersan et al. 2011). The smear zone's lateral and vertical extent should be defined and considered when designing the delivery strategy. If a relatively large portion of the contaminant mass exists as

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[8] Although LNAPLs are by definition less dense than water, sometimes they exist below the water table. For instance, an LNAPL may become trapped beneath the water table by a rising water table.

residual NAPL that is vertically smeared above and below the water table, EAOB may not be able to achieve the site cleanup goals within a reasonable period. This is due to difficulties in effectively supplying amendments to the portion of the smear zone above the water table at the time of injection. However, in such a case, EAOB may still be used at the fringes of the contaminated area while a more aggressive technology, such as multi-phase extraction, is employed in the NAPL zone.

Lateral Extent, Thickness, and Depth

Most of the cost associated with bioremediation are attributable to the methods and time needed to deliver the amendments to the targeted treatment areas, the costs of the amendments themselves, and labor costs associated with monitoring and reporting. The lateral extent, the thickness of the affected zone, and the total depth required to reach the contaminated areas strongly influence the selection of a remedial approach. Target treatment areas that are limited in horizontal and vertical extent with low contaminant concentrations and limited potential for rebound may be ideal for direct injection and will have relatively low implementation costs.[9] Conversely, target treatment areas that are expansive in horizontal and vertical extent with high contaminant concentrations and a high potential for rebound will likely require permanent injection wells with multiple screen intervals, multiple amendment applications, and will have significantly higher implementation costs.

Contaminant Mass Flux and Mass Discharge

Mass flux is the flow rate of contaminant mass through a defined area, usually a portion of a plume cross section. Mass flux is expressed as mass per time per area. Mass discharge is the integration of mass flux measured across an entire plume and thus represents the total mass of any contaminant plume conveyed by groundwater through a defined plane. Mass discharge is expressed as mass per time. In addition to defining the source strength and plume attenuation rate, mass flux estimates can identify areas of the plume’s cross-section where most of the contaminant mass is moving. Mass flux and mass discharge can be measured using transect methods, where concentration and flow data are collected from new or existing monitoring points and integrated; well capture and pump tests, where groundwater is extracted from wells while flow and mass discharge are measured; and passive flux meters, which are instruments that estimate mass flux directly within wells (ITRC 2010).

Incorporating mass discharge information into the conceptual site model will help improve remediation efficiency and may shorten cleanup time, particularly at sites with multiple source areas or where plumes cross multiple stratigraphic units. At some sites, the majority of contaminant mass can flow through a small portion of a cross-sectional area of an aquifer. Guilbeault and others (2005) studied three sites in North America using cross-sectional transects and found that 75 percent of contaminant mass discharge occurs through 5 to 10 percent of the plume cross-sectional area. Mass flux and mass discharge are extremely useful parameters to consider in designing an amendment delivery system, though the cost to collect the data needed to calculate mass flux and mass discharge increases with desired accuracy. The added costs may be justified if there is a possibility that the target treatment area

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[9] See the Aquifer Matrix Diffusion Potential section for discussion of rebound.

could be reduced or more accurately located and addressed. More targeted treatment can reduce overall costs and lead to more effective remediation.

High-Resolution Site Characterization (HRSC)

Effective implementation of remedial technologies, especially in-situ methods such as in-situ bioremediation, requires accurate site characterization. In particular, the use of HRSC can vastly improve the conceptual site model. HRSC has become more prominent as sampling techniques, data evaluation, and presentation methods have improved. HRSC strategies and techniques use scale-appropriate measurement and sample density to define contaminant distributions and the physical context in which they reside with greater certainty, and to estimate the mass flux, supporting faster and more effective site cleanup (CLU-IN 2013). The data obtained from HRSC are used in conjunction with mathematical and visual modeling tools to develop an accurate conceptual site model by identifying heterogeneities in the subsurface that significantly influence contaminant distribution, fate, and transport. These heterogeneities can occur at very small scale that conventional investigation strategies and technologies (primarily placing monitoring wells at biased locations to delineate extent of contamination) can miss. Characterizing sites using HRSC will increase data density and reduce uncertainty.

HRSC uses transects of vertical subsurface profiles oriented perpendicular to the direction of groundwater flow. Profiles located along each transect are used to collect high-resolution lithologic, hydrogeologic, and contaminant data using direct sensing tools implemented using direct push technology (DPT). Lithologic data are collected using such technologies as cone penetrometer testing (CPT), various electrical conductivity (EC) probes, and hydraulic profiling tools. Real-time hydraulic profiling tools provide the best hydrogeologic data.

Several software programs are available to perform three-dimensional visualization and analysis of site characterization and performance monitoring data. These programs are useful for designing amendment delivery systems and identifying the portions of a plume that may require additional amendments. Some practitioners are combining in-situ sensors, for characteristics such as pH, dissolved oxygen, oxidation-reduction potential, chloride, and conductivity, with a Web-based interface to facilitate continuous monitoring and evaluation of additional amendment needs.

Investigation using high-resolution site characterization strategies and technologies should be considered in areas with potential NAPL. The presence or absence of NAPL, and better understanding of the relative thickness and distribution of NAPL, can make order-of-magnitude differences in the total aquifer contaminant mass.

Concentration And Toxicity

Although microorganisms are able to adapt to the conditions of their environment to some extent, very high contaminant concentrations may inhibit the growth of biodegrading microorganisms. In addition, if there is a very large mass of contamination, the biodegrading microorganisms may not be able to address the contaminant mass in a reasonable timeframe. When considering the feasibility of EAOB, you should evaluate the mass of the target contaminants of concern relative to potential biodegradation rates and the cleanup timeframe objective.

The guidance threshold values summarized in Exhibit XIV-9 can be compared to average site concentrations provided in the CAP as a way of forecasting the potential effectiveness of EAOB. Again, it is important to recognize that the values shown in Exhibit XIV-9 are guidance values only.

If petroleum constituents exist at the site above the threshold concentrations in Exhibit XIV-9, this could also indicate the presence of NAPL in the subsurface. In Step 1, the initial effectiveness screening of EAOB, one of the requirements discussed was the absence of NAPL. If threshold soil petroleum levels exist, then NAPL most likely exists in the soil, and EAOB will likely not be effective without first removing the product through other remedial measures. You should also determine whether the site has high concentrations of metals, which could inhibit microbial growth.

Exhibit XIV-9
Constituent Concentration And EAOB Effectiveness

Contaminant Levels (ppm)	EAOB Effectiveness
Petroleum constituents < 50,000	Possibly effective
Petroleum constituents > 50,000	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
Note: Source: EPA 2004, Chapter XII	

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, such as NAPL. 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. Constituents that are highly soluble have a tendency to dissolve into the groundwater and are more available for biodegradation. Conversely, chemicals that have low water solubilities tend to remain in the adsorbed phase and will biodegrade more slowly. The solubility values for petroleum hydrocarbons vary significantly – over four orders of magnitude – as shown in Exhibit XIV-10.

When multiple compounds are present, such as at a petroleum release site, effective solubility values are typically lower.[10] The solubility values in Exhibit XIV-10 are for selected individual chemicals. For example, benzene dissolved in water by itself with no other compounds present can reach a maximum concentration in water of about 1,790 mg/L before a separate phase develops. While not representing effective solubility concentrations that may exist at particular petroleum release sites, the values

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[10] See EPA's effective solubility calculator at https://www3.epa.gov/ceampubl/learn2model/part-two/onsite/es.html.

present in Exhibit XIV-10 provide a sense of the relative solubility concentrations for a range of fuel components.

Exhibit XIV-10
Solubility Values For Select Petroleum Hydrocarbon Constituents

Compound	Solubility in water (mg/L)	Soil organic carbon partition coefficient (K_{oc}) (mL/g)
MTBE	51,000	12
Benzene	1,790	58
Toluene	530	130
Ethylbenzene	210	220
Xylenes (total)	175	350
Cumene	50,000	2,800
Naphthalene	31	950
Acenaphthene	3.5	4,900

Note:
Solubility values are for standard temperature and pressure.

Although low solubility reduces the availability of a contaminant for biodegradation, it also limits the ability of a contaminant to migrate with groundwater. Therefore, bioremediation of low-solubility 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 at shallow depths 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 thus complicating bioremediation. We describe this concept in more detail below.

Soil Sorption

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 greater mass of soil relative to groundwater in a contaminated area, can lead to hydrocarbon mass distributions that are very uneven, making 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. Therefore, it is important to know how the target organic petroleum compounds are partitioned among the various phases (dissolved, sorbed in the unsaturated zone, or sorbed in the saturated zone).

A compound's soil organic carbon partition coefficient (K_{oc}) is a constant that describes the equilibrium condition between organic carbon and the contaminant concentrations in an aqueous solution. Higher K_{oc} values indicate more contaminant mass is likely to be retained in soil and therefore less readily bioavailable. Conversely, lower K_{oc} indicate lower contaminant concentrations will exist in equilibrium in soil for given concentrations in groundwater. Exhibit XIV-10 provides petroleum constituent K_{oc} values for common petroleum hydrocarbons. A comparison of the solubility and K_{oc} values for the sample group of petroleum hydrocarbons reveals the inverse relationship between the two parameters. Compounds with higher solubility values have lower K_{oc} constants.

Special Considerations For MTBE And Other Fuel Oxygenates

The CAP should establish whether fuel oxygenates such as MTBE, tertiary butyl alcohol (TBA), ethanol, or methanol are present at the site. The presence of these fuel additives at a site can affect the rate at which other contaminants biodegrade. Generally, fuel oxygenates are highly soluble in water and highly mobile in the subsurface, allowing them to spread faster and farther than other petroleum hydrocarbons.

Methanol and ethanol are highly biodegradable. Their preferential biodegradation tends to delay the biodegradation of less-preferred substrates such as petroleum hydrocarbons (Farhadian et al. 2008). During this delay, microbial biodegradation may deplete the supply of essential co-nutrients and electron acceptors. Therefore, additional amendments may need to be added over a longer period to achieve the desired remedial objectives for petroleum hydrocarbons if high concentrations of methanol or ethanol are present.

MTBE and TBA are sometimes less biodegradable than petroleum hydrocarbons. However, research has shown that, under certain conditions, MTBE can be biodegraded anaerobically (EPA 2000, Kolhatkar et al. 2000, Kolhatkar et al. 2002, Kuder et al. 2005, Lunardini and Dickey 2003, Mackay et al. 2007, Mormile et al. 1994, Wilson and Kolhatkar 2002, Wilson et al. 2005a). However, not all sites have indigenous microbial communities capable of degrading MTBE. As such, when EAOB is to be utilized for addressing MTBE, it may be prudent to verify that the native microbial population is capable of degrading MTBE before implementing a costly and complex EAOB plan. This can be done with standard microcosm, molecular, or stable-isotope tests. Such laboratory tests can also be used to optimize the EAOB procedures for the site to ensure 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 the specific microbes desired for MTBE biodegradation. When bioaugmenting with a cultured microbial suite, it is important to understand its technical effectiveness and cost effectiveness, and the longevity of the microbes. Due to the vagaries of geochemistry and microbiology in the subsurface, site-specific microcosms 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). This constituent of concern often rapidly disappears from the subsurface at some biodegradation sites, while at other sites the TBA seems to be recalcitrant. You should be aware of the possible subsurface creation of TBA, and seek to avoid creating a 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 percent by volume, and it is often found in commercial-grade MTBE at 1 to 2 percent 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 EAOB activities can help to determine if the TBA was in the original gasoline spill or if it is present due to biodegradation of MTBE. 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.

Before you consider using EAOB for treating MTBE, review the list of questions presented in Exhibit XIV-11.

Exhibit XIV-11
MTBE Considerations For Applying EAOB

- 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?
- If there is a need to treat a larger area or greater depth, does this require installing more electron acceptor 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 and, if so, will it be effective in-situ?
- Has the greater mobility of the MTBE been accounted for in the plan?
- Does the presence of more readily biodegradable compounds (e.g., 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 MTBE? Does the site contain a sufficient amount of electron acceptors and appropriate microbial suite (native or bioaugmented)?
- Has the CAP accounted for the possible biological formation of the intermediary product TBA, including the possibility of creating a TBA plume?

Treatability Testing

Treatability studies are often performed during or after development of remedial alternatives (feasibility study) when bioremediation is a potential site remedy. Treatability studies are used to further evaluate whether the proposed bioremediation remedy will be successful under site conditions. Treatability studies must take into account the site's heterogeneity in site conditions and contamination. Treatability studies generally include two phases: bench testing and pilot testing. Bench tests, often referred to as microcosm studies for anaerobic projects, are conducted in the laboratory to evaluate the feasibility of a process under idealized or specifically controlled conditions, while pilot tests simulate full-scale operations and are often conducted in the field where localized conditions may vary. These studies also provide the design information required for full-scale implementation. Successful microcosm results do not guarantee successful implementation results at the pilot or full scale. Once designed and installed, a bioremediation system will require process and performance monitoring and possible modification to optimize the bioremediation system.

Bench testing is typically performed on a sample of the site soil, groundwater, and bedrock, if present, collected for use in laboratory-scale treatment studies. Bench tests are generally used to evaluate:

- The performance of various amendments

- Substrate demand and loading rates that can subsequently be tested in a pilot test
- Whether addition of bacterial culture is needed or is likely to succeed
- The optimal consortium or combination of bacterial cultures
- The relative rate of biodegradation that may be achievable under certain conditions
- The treatability of contaminants at different concentrations

Bench testing allows for easier manipulation and testing of many variables. If site conditions are favorable for a particular bioremediation approach, the cost of a bench test may outweigh the benefits. However, if site conditions are marginal, a bench test could be useful in evaluating whether bioremediation can be applied at a site before additional investment is made in pilot testing. Bench testing may not always accurately reflect subsurface conditions in the field; however, downhole forms of microcosms, called Bio-Traps®, can also be used in the field as an alternative to bench studies. A Bio-Trap® is a commercially available passive sampling tool containing a matrix that encourages colonization by subsurface microbes. Bio-Traps® can be used to test different amendments and microbe consortiums on a microcosm scale and calculate degradation rates (EPA 2013).

Pilot tests are usually small-scale field tests and typically include a set of injection wells or direct injection points and monitoring wells at varying distances and depths within the pilot test treatment area. Monitoring wells may be positioned radially around the injection area when groundwater flow velocities are low, or at various downgradient distances when groundwater flow velocities are naturally higher or a recirculation cell will be established. When possible, a tracer test using a conservative tracer, such as an ion salt (such as sodium bromide) or a dye (for example, fluorescein or rhodamine), should be completed as part of the start of the pilot test to help determine groundwater flow paths, dispersion, effective porosity, and velocity (EPA 2013). Estimating porosity and groundwater velocity reliably through tracer testing is important for successful application of EAOB.

The results from pilot tests will help identify microbial response to biostimulation and provide design data regarding the injection wells' radii of influence and the performance of the amendment. Pilot test results are used to establish the full-scale injection well spacing and depth interval, quantity of amendment, and the frequency of application. If bioaugmentation is planned, pilot tests should be used to evaluate the likelihood of success. A pilot test is generally designed with scalability in mind. For example, the designer should consider whether a given pilot test layout could be scaled up to treat the total target area within the cleanup and cost parameters of the project. In addition, a designer should decide whether they need to consider other factors, such as source water and electricity demands for pumps that may be in more remote locations during full-scale implementation. In some cases where bioaugmentation is not planned, a bench test will not be required if a pilot test is well designed (EPA 2013).

A pilot test may examine the effectiveness of multiple amendments, nutrients, and bacterial cultures, rather than simply confirming the results of the bench test. However, pilot testing of multiple amendments or cultures requires some design considerations to differentiate results. One design would be to test various amendments or cultures in different portions of the pilot test area, separated by an unamended control area. Another design would be to test amendments sequentially (EPA 2013).

Pilot tests should be conducted for a period long enough to determine if complete biological degradation is likely, in addition to obtaining the design data previously mentioned. However, longer pilot tests could provide important information regarding amendment longevity, longer-term microbial and aquifer geochemical responses, potential maintenance issues, and contaminant rebound characteristics (EPA 2013).

Complex and highly heterogeneous sites often involve several target treatment zones. Each treatment zone may require unique delivery and amendment designs, depending on the differences in hydrogeology, depth, or co-contaminants. As a result, a successful pilot or bench study directed at one zone does not guarantee success for the other zones. Sites with dissimilar target treatment zones could become more expensive to treat than expected if this level of detail is not addressed at the site characterization and feasibility study stages of a project.

Evaluation Of Enhanced Anaerobic Oxidative Bioremediation Design

This section provides guidance on reviewing and evaluating the EAOB design. It focuses on prompting reviewers to identify and review key elements of the CAP to help ensure that it demonstrates a coherent understanding of the basis for the EAOB system design. In addition, this section provides information on typical in-situ bioremediation technology components to help verify that the CAP has included the basic equipment requirements for the remedial system.

We assume that the detailed technology screening process, as described in Steps 1 and 2, verified that EAOB appears to be appropriate and is expected to be an effective cleanup approach given site-specific conditions. If you have not completed the EAOB effectiveness evaluation, we strongly recommend that you do this before you evaluate the design.

Design Basis

Review of the CAP should find consistency between site characterization work and information presented as the basis for the EAOB design. To conduct the EAOB effectiveness evaluation, you should understand the nature and extent of the site-specific petroleum constituents of concern, the contaminant phases present, and the relevant site chemical, physical, and biological properties. Additionally, you should ascertain when and to what extent the groundwater resource might be needed, for example, public and private drinking water supplies; this will inform the rate and extent of remediation required. When preparing and reviewing the CAP design, you should 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 general foundation 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 CAP 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.

After a review of the site characterization, additional information is often needed to complete the remedial system design. The CAP should include the results and interpretation of the follow-up studies completed after the original site characterization. For example, the site characterization may suggest that one or more of the constituents of concern is considered marginally biodegradable, and that you will need additional data to predict the level of expected biodegradation.

Exhibit XIV-12 summarizes examples of information expected to be developed during the site characterization, or as a result of follow-up studies, to support the basis for the technology selection and design of the CAP.

Exhibit XIV-12 EAOB Design Basis Factors	
Design Basis Factor	**Sources Of Design Information**
Cleanup Goals - Target contaminant levels (soil and groundwater) - Remediation timeframe - When and to what extent is groundwater needed for drinking water supply	- Receptor survey, pre-design exposure or risk assessment analyses (potentially including numerical modeling), state requirements, and state water resource boards
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) - Groundwater temperature - Modeling results	- Site characterization well gauging, aquifer pump testing, tracer test, pilot test, 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) and heterogeneity - 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

The cleanup goals that the remediation program must achieve influence the evaluation of alternative remedial approaches and the subsequent design of the selected approach. 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, you should ensure that the cleanup goals are fully evolved and solidified in the CAP.

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 or all of the following:

- Health-based numeric values for petroleum chemical constituents published by the respective regulatory agency.
- Cleanup goals developed and proposed by the responsible party specifically for the contaminated site that are acceptable to the regulatory agency.
- Risk-based cleanup goals derived from site-specific risk assessment involving contaminant fate and transport modeling coupled with ecological and human-health risk assessment
- Generic state or federal 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 CAP. Regardless of what the cleanup goals are and how they are established, the regulatory agency's goals should be noted in the CAP and recognized as a fundamental basis for the technology selection and design.

Cometabolism occurs when microorganisms using one compound as an energy source fortuitously produce an enzyme that chemically transforms another compound. Cometabolic bioremediation may prove useful in reducing concentrations of some contaminants. Organisms can degrade a contaminant without gaining any energy from the reaction. Cometabolic degradation is a process that often happens concurrently in bioremediation systems designed for direct metabolism of contaminants; however, some systems have been designed to specifically take advantage of cometabolic processes. Hazen (2009) indicates that cometabolic bioremediation can occur in environments where contaminant concentrations are well below concentrations that could provide a carbon or energy benefit to the biodegrader. Therefore, this method may be effective at degrading very low concentrations of some contaminants. There is laboratory evidence of anaerobic cometabolic degradation of hexachlorocyclohexane, BTEX, polycyclic aromatic hydrocarbons, atrazine, and trinitrotoluene (TNT), though these remedies have not been used extensively in the field (EPA 2013).

While further bioremediation of petroleum contaminant levels in the subsurface may become limited at some point due to the limited availability of a usable carbon source, the target chemical constituents that 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.

Technology Selection

The primary decisions when selecting an EAOB technique are which electron acceptor(s) and other amendments to apply and what type of distribution system to use.

Selection Of Electron Acceptors

Research on EAOB for petroleum has focused on the use of nitrate, sulfate, and iron as electron acceptors. These compounds are often naturally present in aquifers. Various commercial products are available that can supply electron acceptors to drive the anaerobic oxidation process. These products most commonly contain iron(III), nitrate, or sulfate. Less research has focused on the use of manganese and carbon dioxide as electron acceptors.

The selection of an electron acceptor or product will depend on the contaminant, current site conditions, and the preferred oxidation-reduction state of the targeted bioremediation process, seen in Exhibit XIV-5. As shown in Exhibit XIV-13, nitrate, iron, and sulfate have strong advantages as electron acceptors over carbon dioxide and manganese, given that nitrate, iron, and sulfate have a documented ability to facilitate the degradation of all BTEX compounds. As shown in Exhibit XIV-14, you can deliver nitrate and sulfate to groundwater at higher concentrations than can oxygen and iron, allowing a greater mass of BTEX to be biodegraded. Exhibit XIV-14 presents the practical concentration limits for the various electron acceptors; these limits are determined by solubilities, regulatory restrictions, and the need to avoid undesirable effects. Note that, by replenishing the supply of oxygen, enhanced aerobic oxidation systems generally allow for more rapid biodegradation of reduced contaminants than do anaerobic systems. Exhibit XIV-15 presents the advantages and disadvantages of various electron acceptors.

You should ensure that the type and concentration of electron acceptor proposed for use would not adversely affect drinking water aquifers. For example, nitrate has a federal health-based limit on concentration in drinking water. Manganese, iron, and sulfate have federal limits on concentration in drinking water based on aesthetic or technical effects, such as color, taste, and staining. CAP reviewers should also check whether state or local standards limit the allowable concentration of electron acceptor.

Exhibit XIV-13
Anaerobic Biodegradability Of Selected Petroleum Constituents Using Various Electron Acceptors

	Electron Acceptor				
	Nitrate	**Manganese**	**Iron**	**Sulfate**	**Carbon Dioxide**
Benzene	X		X	X	X
Toluene	X	X	X	X	X
Ethylbenzene	X		X	X	
Xylenes	X		X	X	

Notes:
X = anaerobically biodegradable using this electron acceptor
Sources: Chakraborty and Coates 2004; Jahn et al. 2005

Exhibit XIV-14
BTEX Mass That Can Be Biodegraded By Various Electron Acceptors

Electron Acceptor	Maximum Practical Concentration In Groundwater (mg/L)	Reasons For Limiting Concentration In Groundwater	BTEX Degraded (mg/L)[a]
Oxygen	9-10	Aqueous solubility; aquifer clogging from biomass formation or from oxidation of Fe(II) to Fe(III)	2.9-3.2
Nitrate	80-100	Formation of N_2 gas bubbles in-situ; drinking water limit of 45 mg/L (equal to the federal primary maximum contaminant level of 10 mg/L measured as nitrogen)	16-21[b]
Iron	0-1	Fe(III) salts have very low solubilities; aquifer clogging from precipitation of iron sulfide	0-0.05
Sulfate	100-250	Formation of inhibitory sulfide; secondary drinking water limit = 250 mg/L (based on taste in drinking water)	21-53

Notes:
a) BTEX degradation is calculated as toluene degradation, and assumes entire mass of electron acceptor is used to degrade toluene.
b) At nitrate's drinking water limit of 45 mg/L, the BTEX degraded would be 9 mg/L.
Source: Cunningham et al. 2001

Several microbes involved in bioremediation can adapt to both aerobic and anaerobic conditions; these microbes are described as being facultative. Although facultative microbes can be active in both aerobic and anaerobic environments, they degrade contaminants at a slower rate in the absence of oxygen. Microbes that use nitrate as an electron acceptor tend to be facultative (Firestone 1982). Strict anaerobes will be active only in reduced-oxygen environments and will use electron acceptors such as sulfate or carbon dioxide. Sulfate-reducing bacteria are obligate anaerobes, as they require an anaerobic environment to thrive.

Exhibit XIV-15
Advantages And Disadvantages Of Various Electron Acceptors

	Advantages	**Disadvantages**
Nitrate	• High solubility • Capable of anaerobically biodegrading all BTEX compounds	• Regulatory limit on concentration in drinking water based on health effects
Manganese		• May not be able to anaerobically biodegrade some BTEX compounds. • Aesthetic limit on concentration in drinking water based on color, staining, and taste
Iron	• Capable of anaerobically biodegrading all BTEX compounds	• Very low solubility • Aquifer clogging from precipitation of iron sulfide • Aesthetic limit on concentration in drinking water based on color, staining, taste, and sediment
Sulfate	• High solubility • Capable of anaerobically biodegrading all BTEX compounds	• Potential to produce toxic hydrogen sulfide gas • Aesthetic limit on concentration in drinking water based on taste
Carbon dioxide		• May not be able to anaerobically biodegrade some BTEX compounds fast enough for project needs

Sulfate

In natural or unenhanced systems, sulfate reduction is the main pathway for biodegradation of petroleum contamination, "…accounting for approximately 70% on average of the natural biodegradation capacity…" (Suthersan et al. 2011). Suthersan et al. (2011) list several important advantages of using sulfate as an electron acceptor in an EAOB project:

- Sulfate-reducing bacteria are able to biodegrade many classes of petroleum hydrocarbons

- Sulfate is highly soluble in water and tends not to sorb onto soil particles, allowing large quantities of electron acceptor to be made available to biodegrading microbes

- Sulfate amendments are persistent in the subsurface, remaining available for use by biodegrading microbes for an extended time

- Sulfate amendments are commercially-available in bulk quantities from remediation product suppliers at relatively low cost

Large numbers of sulfate-reducing microbes are naturally present in many subsurface environments. Suthersan et al. (2011) note that sulfate-reducing microbes can be stimulated within 45 days after the introduction of sulfate amendments, even at sites where little or no sulfate reduction was occurring before the addition of sulfate, showing that "…sulfate-reducing organisms are ubiquitous and robust in terrestrial systems..." *Desulfovibrio* is the most well studied sulfate reducer.

CAPs that propose to use sulfate should describe plans for dealing with several potential concerns that can arise when using sulfate amendments. Most of these concerns can be managed by controlling the amounts and rates of sulfate that are delivered. The concerns include:

- Secondary drinking water limit for sulfate (250 mg/L)
- Potential to create hydrogen sulfide gas as a byproduct
- Potential to increase alkalinity and total dissolved solids

The federal secondary drinking water limit for sulfate is 250 mg/L, due to a salty taste when sulfate is present at higher levels. Suthersan et al. (2011) conclude that "…generally, the recommended minimum threshold sulfate concentration to sustain treatment is approximately 100 mg/L … Sulfate concentrations in excess of approximately 2000 mg/L are not expected to yield increased PHC [petroleum hydrocarbon] oxidation rates."

The following formula describes the degradation of benzene, with sulfate acting as the electron acceptor:[11]

$$C_6H_6 + 3.75SO_4^{2-} + 3H_2O \rightarrow 1.875H_2S + 1.875HS^- + 6HCO_3^- + 0.375H^+$$

As shown in the above formula, sulfate reduction produces sulfide (H_2S and HS^-). Iron, which is naturally present in most aquifers, reacts with the sulfide to form a precipitate, iron sulfide. Sulfide also reacts with other dissolved metals to form a precipitate, thereby reducing the mobility of the metals. Theoretically, if there is not enough iron or other metals in the subsurface to precipitate the sulfide, or in acidic conditions, hydrogen sulfide gas would be generated, which is toxic and flammable. This could cause vapor intrusion concerns, depending on whether buildings are present near the treatment area. However, problems with hydrogen sulfide generation rarely happen in the field (Suthersan et al. 2011). In addition to the natural protection afforded by iron precipitation, oxygen and nitrate rapidly oxidize sulfide, limiting the persistence of sulfide in the subsurface.

An additional problem that could arise if excess concentrations of sulfides accumulate is the inhibition of sulfate-reducing bacteria, which would reduce the rate of biodegradation. The presence of iron in the

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[11] Suthersan et al. 2011

aquifer minerals usually prevents this problem from arising. In addition, the CAP should ensure that sulfate dosing would be controlled, in order to reduce the potential for excessive sulfide accumulation.

The chemistry of sulfate reduction can raise the groundwater's pH over time, due to the production of bicarbonate. Increases in pH above 9 have been observed at EAOB sites with limited buffering capacity (Suthersan et al. 2011). High pH can lead to precipitation of calcium carbonate, causing reduced aquifer permeability.

Suthersan et al. (2011) describes two additional issues that should be considered when designing a sulfate-based EAOB system. First, the delivery of soluble sulfate salts will increase the concentration of total dissolved solids (TDS). The increase in TDS can cause injection solutions to migrate downward due to their greater density, which should be considered in the delivery design. Second, in calcium-rich aquifer systems, sulfate injection can lead to precipitation of gypsum. This can temporarily clog the aquifer, complicating the ability to conduct future injections.

Nitrate

Nitrate is another effective electron acceptor used in EAOB. The use of nitrate has several advantages, as well as significant disadvantages, as shown in Exhibit XIV-15. Nitrate-reducing microbes are capable of biodegrading all BTEX compounds. In addition, nitrate is highly soluble, mobile, and persistent in groundwater, making it possible to deliver large amounts for use by nitrate-reducing microbes. As described in the Terminal Electron Acceptors section starting on page XIV-16, nitrate is the most preferred anaerobic electron acceptor, because nitrate reduction is the metabolic pathway that provides the most energy to microbes after oxygen has been depleted.

The main disadvantage to the addition of nitrate is its toxicity to human and ecological health. Due to its toxicity, nitrate has a federal drinking water standard (10 mg/L measured as nitrogen, which is equivalent to 45 mg/L measured as nitrate). This standard is a federal primary maximum contaminant level based on health, as opposed to the secondary standard for sulfate, which is based on taste. Due to nitrate's negative health effects, some states have regulatory prohibitions against injecting nitrate into groundwater. If a CAP proposes to introduce nitrate into the subsurface, you should verify whether the state allows nitrate injection. If the state allows nitrogen injection, you should ensure that the plan intends to use no more nitrate than is needed to achieve effective bioremediation. Nitrate dosing should be constantly monitored and should attempt to maintain constant, low levels of nitrate, rather than allowing nitrate levels to fluctuate. Slow-release sources of nitrate may be better able to avoid fluctuations in concentration. The CAP should include plans for frequent monitoring of groundwater nitrate levels to ensure that concentrations remain acceptable.

Depending on the type of microbial nitrate reduction occurring at a given site, excessive additions of nitrogen can cause soil pH to decrease; conversely, if the nitrate is reduced to ammonia rather than N_2, soil pH will increase.

The following formula describes the degradation of benzene, with nitrate acting as the electron acceptor (Farhadian et al. 2008):

$$C_6H_6 + 6NO_3^- + 6H^+ \rightarrow 6CO_2 + 6H_2O + 3N_2$$

Nitrate is reduced to nitrite, nitric oxide, nitrous oxide, nitrogen gas, or a combination of these byproducts, depending on the microbes that are present. Nitric oxide, nitrous oxide, and nitrogen gas are gaseous byproducts that can dissolve into groundwater to some extent, but will generally escape into the vadose zone; however, the gaseous byproducts may become trapped within pore spaces, displacing water and reducing the hydraulic conductivity of the saturated matrix.

Use Of Multiple Electron Acceptors

Some proprietary products provide multiple electron acceptors. Cunningham et al. (2001) suggested the combined application of multiple electron acceptors to increase the total electron accepting capacity and to biodegrade as many classes of petroleum contaminants as possible. By injecting both nitrate and sulfate, Cunningham et al. achieved a total electron accepting capacity greater than would be possible with nitrate or sulfate alone, given the limitations on the concentrations of nitrate or sulfate that can be introduced to groundwater, as seen in Exhibit XIV-14. The researchers found that microbes rapidly utilized the injected nitrate; microbes only utilized sulfate in areas where nitrate was not present. Therefore, the researchers created a nitrate-reducing zone near the injection point, and created a sulfate-reducing zone farther away from the injection point.

Although nitrate was used preferentially over sulfate, sulfate reduction was the primary mechanism responsible for the biodegradation of xylenes. This finding indicates that introducing multiple electron acceptors may more effectively biodegrade multiple classes of contaminants.

Although the Cunningham et al. experiment was conducted anaerobically, the researchers stated that practical applications of in-situ bioremediation should also include the application of oxygen, to maximize oxidation of contaminants. This would create an aerobic zone closest to the injection point, surrounded by a nitrate-reducing zone, and finally a sulfate-reducing zone on the periphery.

Iron

Iron may be useful when introduced in combination with other electron acceptors. However, there are strong disadvantages to the introduction of iron alone as the sole electron acceptor. Iron(III) salts are only slightly soluble in water, but when used as an electron acceptor, iron(III) is reduced to iron(II), which is much more soluble in water. Iron(III) has a particularly low electron accepting capacity for its mass, and therefore iron(II) may quickly exceed water quality thresholds in groundwater as it reacts and dissolves.

Delivery Methods

The key concern when an anaerobic bioremediation system is designed is the availability of electron acceptors and nutrients. The amendment delivery system must deliver the amendments to the entire contaminated area of the aquifer.

EAOB projects typically deliver amendments to the subsurface in a solution using injection wells or direct-push injection tooling. For more information on direct-push technology, refer to "*Expedited Site Assessment Tools for Underground Storage Tank Sites: A Guide for Regulators," Chapter V Direct Push*

Technologies.[12] Exhibit XIV-16 shows three common application methods: injection wells, permeable reactive barrier (PRB), and recirculation. Biological groundwater amendments have also been applied using infiltration trenches. Successful delivery requires matching the proper concentration of solution to the remedial objective and avoiding potential negative impacts to the groundwater. Exhibit XIV-17 is a plan view of several treatment configurations.

Both with barrier systems and with more traditional injection systems, it is important to consider the need for multiple delivery events over time in the design. PRBs may need replenishment and point delivery systems may require multiple injection events to sustain desired in-situ conditions to meet remedial objectives. It is critical to ensure appropriate contact time between amendments, microbial populations, and the contaminants of concern. In addition, the initial designs should consider how PRB-type systems can negatively alter natural groundwater flow patterns, or how installation locations may be overlain by structures in the future, which could prevent access to the installation location.

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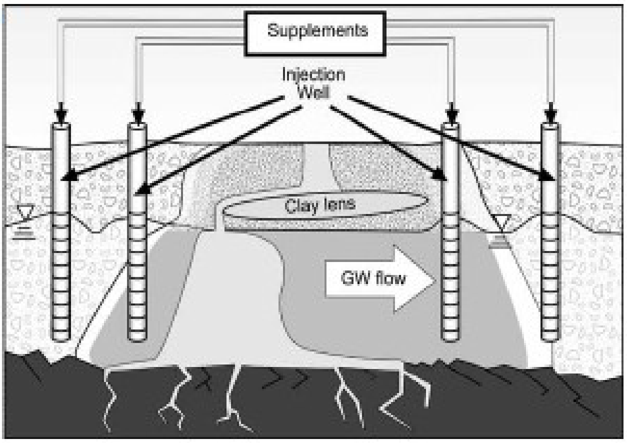
[12] https://www.epa.gov/ust/expedited-site-assessment-tools-underground-storage-tank-sites-guide-regulators

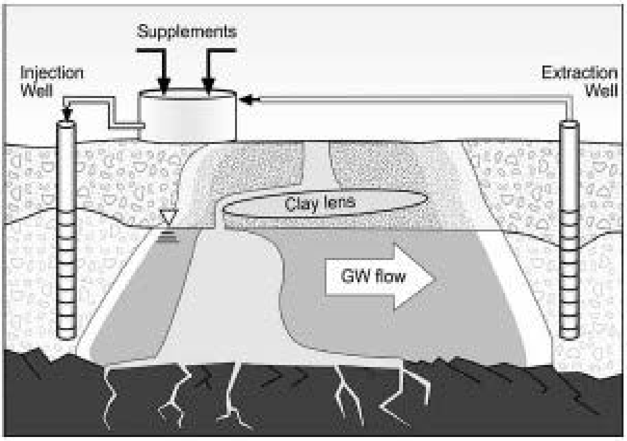
Exhibit XIV-16
Delivery Methods For In-Situ Bioremediation

Active Or Semi-Passive Methods:

Direct Injection

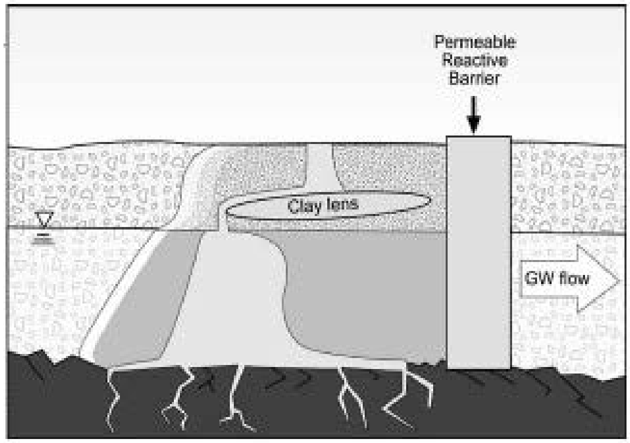


Recirculation



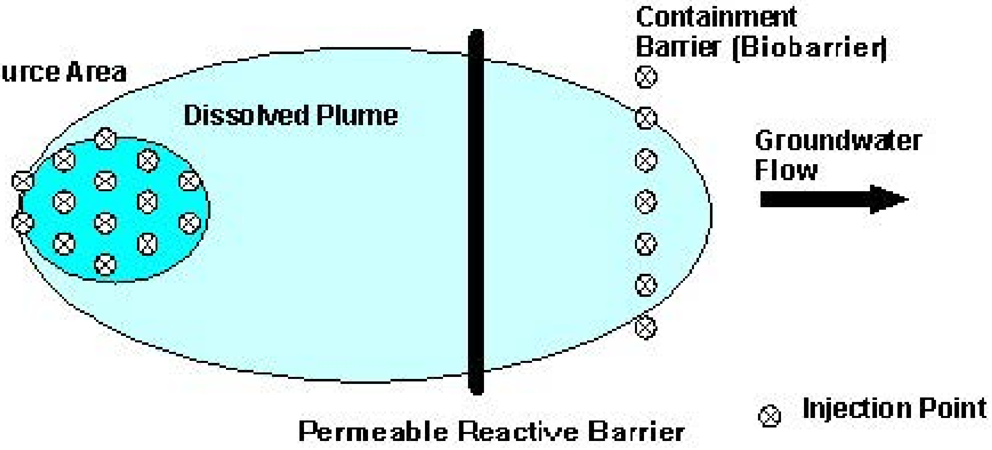
Passive Method:

Permeable Reactive Barrier



Source: EPA 2013

Exhibit XIV-17
Various Treatment Configurations



Source: EPA 2013

There are three primary treatment approaches to full-scale implementation: active, semi-passive, and passive:[13]

- **Active treatment** approaches to bioremediation include circulation of groundwater in the target treatment area. Frequent operation and maintenance (O&M) is required to make system checks and adjustments. Circulation approaches can effectively treat target areas in less time and, as a result, may have lower total life cycle costs. Paired injection and extraction typically increases the hydraulic gradient at a site, thus increasing the rate of distribution and delivery of amendments, but may decrease contact time, which is also a factor in effective remediation. Active treatment is often applied to source areas and highly concentrated, smaller dissolved plumes where elimination of significant mass in a short time can provide the best value to an overall treatment program. Recirculation-type systems often are employed where hydraulic control is a necessary element of the bioremediation program.

- **Semi-passive treatment** approaches to bioremediation are similar to active approaches, but semi-passive systems are not operated continuously. Amendments are circulated throughout the target treatment area in pulses. For example, amendment might be circulated for three months, perhaps long enough to circulate one pore volume within the target treatment area.[14] Circulation is halted, and the site is monitored for a time to determine when additional amendments are required. Semi-passive treatment may take more time than active treatment, but may result in less energy consumption, less O&M, and less use of amendment. As a result,

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[13] EPA 2013

[14] "Pore volume" is the total volume of pore space.

semi-passive treatment approaches may have a lower total life cycle cost than active approaches and are therefore probably the most commonly applied method of bioremediation. One drawback with semi-passive approaches is that there typically is some trial and error to optimize the intervals between pulsed injection events. In practice, two to four injection events over two to five years is common, although this may vary site to site based upon localized site conditions, levels of contaminants, cleanup objective, and other site characterization factors.

- **Passive treatment** approaches, such as injection wells and PRBs, rely on natural flow of groundwater to deliver contaminated groundwater to biologically active areas where treatment occurs. In passive treatment, groundwater is not circulated for extended periods, slow-release amendments are more often used, relatively little infrastructure is required, and treatment times can be longer. PRBs may have certain limitations related to the practical depth at which they can be cost-effectively employed, and may not be feasible in locations with overlying infrastructure. Design of PRBs can be complicated, due to the need to ensure that the amendments come into contact with groundwater and contaminants as desired. Advanced modeling may be required to ensure a successful design.

Determination of amendment quantities, regardless of delivery method, can be calculated based on stoichiometry, estimates of biological demand, or rules of thumb found in guidance documents, literature, or provided by product vendors. Another consideration in calculating amendment quantities is the application rate. Suthersan et al. (2011) state that "…dosing strategies that achieve moderate and more consistent concentrations are best to achieve optimum treatment efficiency and limit secondary geochemical effects." Application rates can be estimated using results from bench testing of various contaminant concentrations; however, the site's geology and hydrogeology are often the limiting factors in determination of injection rates; it may not be possible to achieve a desired bench-scale application rate in the field due to low-permeability soils in-situ or daylighting in shallow aquifer systems. In the absence of bench testing, application of amendments at a uniform rate across a target treatment area still may not be the best approach. A more cost-effective methodology is often to focus the amendment in the horizontal and vertical zones of highest concentrations to address the areas of greatest flux and to reduce the application rate on the margins of the plume. It is important to recognize that the highest concentrations may not be in the most transmissive zones. Various amendment quantities can be identified for different zones and injection infrastructure can be designed for each zone. High-resolution vertical profiling of the aquifer characteristics and contaminant distribution can result in a more precise and targeted design.

Design Components

Although the design elements of EAOB technologies can vary significantly, Exhibit XIV-18 describes some typical design components. We discuss several of the more important elements below to assist with evaluation of the CAP.

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 EAOB 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 sewers, air or soil vapor discharge, or permits for possible chemical storage.

Several federal, state, and local programs regulate Class V aquifer remediation wells, and many require permits for underground injection of bionutrients. 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. Some states and localities have used these authorities, as well as their own authorities, 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 program, operating with approval from the federal program, may have jurisdiction over such wells. Second, in some states without underground injection control programs, the state's program for groundwater protection or pollution elimination program requirements may apply to remediation wells. Third, federal and state authorities may regulate remediation wells through Superfund programs, corrective action programs under the Resource Conservation and Recovery Act (RCRA) including the underground storage tank 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 voluntary cleanup programs, some concern exists because remediation wells may not be approved or completed according to standards typical of cleanups overseen by a state or federal agency.

Performance monitoring should be accounted for in the design in the form of a written plan that can be used to evaluate EAOB system performance in both the short- and long-term. The plan should clearly describe the approaches and methods that will be used to evaluate EAOB system effectiveness in each of the following:

- Delivering electron acceptor (and bio-nutrients) to the subsurface
- Distributing electron acceptor and bio-nutrients through the contaminated area
- Increasing microbial population density
- Reducing sorbed and dissolved phase petroleum concentrations
- Minimizing adverse side reactions (e.g., increases in toxic metals concentrations, etc.)
- 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 problems, including those associated with the remediation system and changing environmental conditions, and outline appropriate response actions. Problems with the remediation system may include:

- Inadequate distribution of electron acceptor
- Stagnation or die-off of microbial populations
- Low petroleum mass reduction rates

- Excessive contaminant migration
- Recalcitrance of constituents
- Biofouling
- Fugitive emissions
- Any other reasonably plausible scenario that can arise under site-specific conditions and project-specific circumstances

Exhibit XIV-18
Suggested EAOB Design Components

- **Electron Acceptor And Bio-nutrient Delivery Design**
  - Theoretical electron acceptor mass requirement
  - Bio-nutrient needs (e.g., nitrogen, phosphorus)
  - Application delivery rate, volumes, and frequency
  - Number, depth, and placement of application points
  - Provisions for future injections and site access
  - Injection and monitoring equipment and associated power and utility needs

- **Permit Requirements And Thresholds**
  - Underground injection/well installation
  - Groundwater discharge
  - Air (soil vapor) discharge
  - Chemical storage

- **Performance Monitoring Plan**
  - Ongoing distribution of electron acceptor and bio-nutrients
  - Expansion of microbial population
  - Reduction in contaminants (sorbed and dissolved phases) and affected elements in the subsurface
  - Formation of daughter constituents as indicator of biodegradation
  - Formation and persistence of byproducts

- **Contingency Plan That Addresses The Following:**
  - Inadequate electron acceptor distribution
  - Stagnation or die-off of microbial population
  - Lower-than-expected petroleum mass reduction rates
  - Excessive contaminant migration
  - Buildup of excessive recalcitrant petroleum constituents
  - Buildup of harmful byproducts
  - Fugitive (soil vapor) emissions
  - Difficult-to-treat/fouling of treated wastewater discharge
  - Clogging of equipment or injection areas with precipitates
  - Other contingencies

Components Of An Enhanced Anaerobic Oxidative Bioremediation System

After review of factors that affect the selection and design of a particular EAOB technology and the critical elements that should be included in the CAP for EAOB, it is appropriate to discuss major components of various EAOB systems.

Exhibit XIV-19 summarizes some of the major equipment components associated with EAOB technologies. The major system components should be presented, discussed, and schematically depicted (e.g., process flow diagram) in the CAP. The design should relate capacities of these equipment components to design requirements.

Exhibit XIV-19
Major Components Of EAOB Systems

Component	Function
• Injection Wells or Infiltration Galleries	• Injection wells, infiltration galleries, or a combination of these are used to inject amended groundwater.
• Extraction Wells	• Often used to extract contaminated groundwater downgradient of the contaminated area for treatment or reinjection in the upgradient source area for plume containment or accelerated groundwater flow through the contaminated area.
• 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 amendments into the infiltration system to maintain design concentrations
• 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
• 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 electron acceptor and petroleum contamination generally indicate how effectively electron acceptor is being delivered or dispersed and whether contaminant reductions are occurring

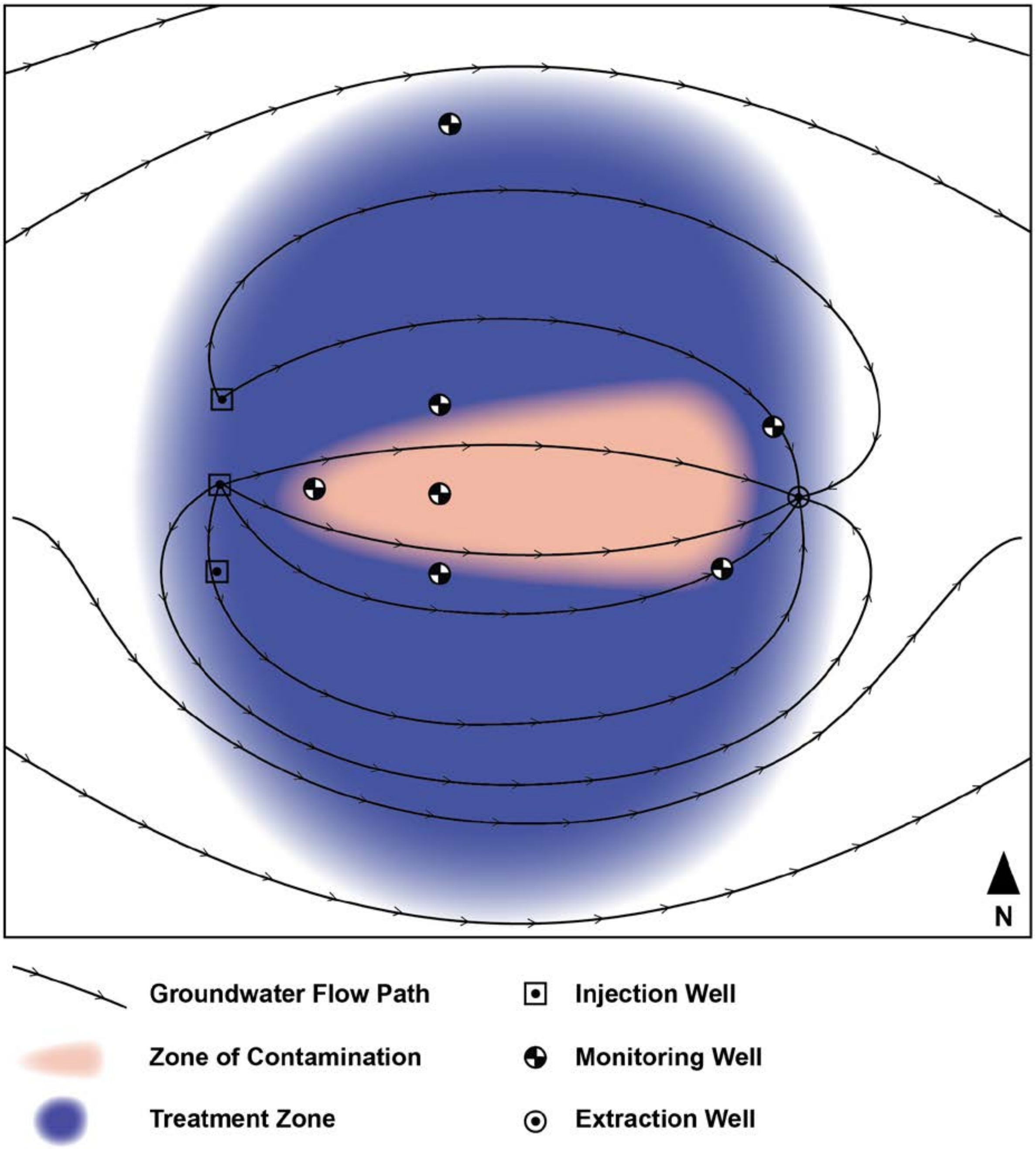
Types And Locations Of Wells

Since the most common electron acceptors used for anaerobic bioremediation are soluble in water, the products are typically delivered to the subsurface in a solution via permanent injection wells or temporary direct injection points. The number and location of wells and injection points are determined during the design to optimize delivery of amendments to the contaminated area. Maintenance applications are sometimes overlooked during the initial design; considering the future drilling costs associated with maintenance applications may indicate that installing permanent wells would be less expensive than using temporary direct injection points. In general, if more than three or four injection events are anticipated, permanent wells may be more economical than temporary driven points in many site applications.

Monitoring wells are also needed to measure the bioremediation system's performance. Wells that are used to carry out remedial actions should not be used as monitoring wells for final remedial performance verification; however, they may be useful in evaluating progress and effectiveness parameters while the remedial program is ongoing. Monitoring wells used for cleanup determination should be separate wells used only for that purpose, in order to most accurately portray the conditions in the aquifer at large rather than at an injection or extraction point.

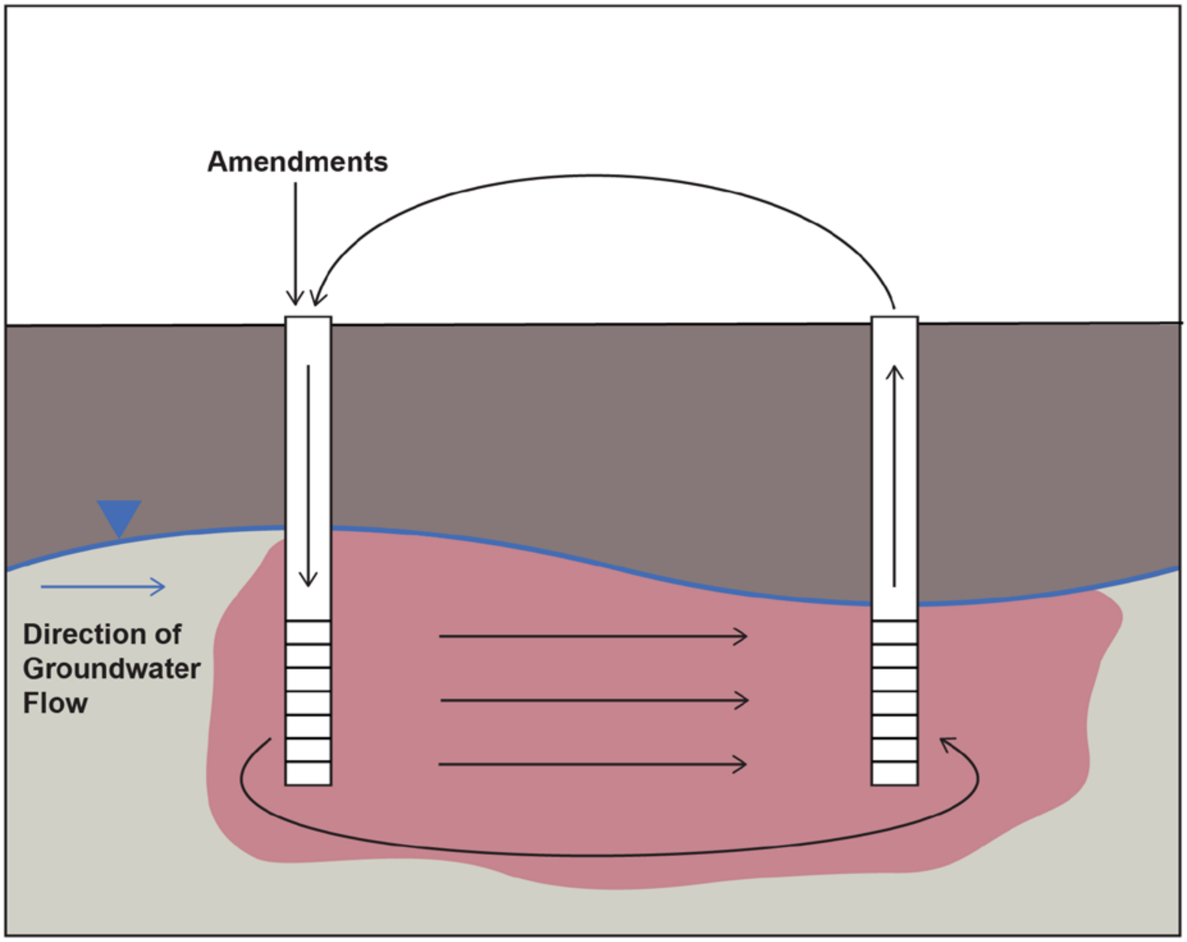
Recirculation systems are one of the most efficient ways to distribute amendments. Recirculation systems typically have extraction wells drawing groundwater from downgradient locations, and injection wells injecting water with amendments into the source area (see Exhibits XIV-20 and XIV-21). This increases the hydraulic gradient and accelerates the distribution of amendments across the contaminated area, which can shorten the time needed for remediation. The responsible party may wish to conduct a site-specific cost-benefit study to determine whether the benefits (for example, less time needed to achieve remedial objectives, decreased long-term O&M costs, or other business factors) outweigh the costs of installing and operating a recirculation system. The number, location, and design of the extraction wells will largely be determined from site-specific hydrogeology, the depth, and thickness of the contaminated area, and the results of field-scale pilot testing and hydraulic modeling. Recirculation allows for greater hydraulic control and manipulation and can be used to create treatment areas beneath buildings, active roads, runways, and other areas with limited site access, while at the same time preventing or limiting off-site contaminant migration potential. The CAP must ensure that the extraction wells will capture the added groundwater gradient created by the injection wells. If not, contaminants may be pushed past the extraction wells and off site.

Exhibit XIV-20
Recirculation System Layout



Adapted from EPA 2013

Exhibit XIV-21
Schematic Of A Horizontal Recirculation System



Adapted from Air Force Center for Environmental Excellence et al. 2004

Treatment area grids can be used to address source areas and smaller dissolved plumes in groundwater. Treatment areas are often implemented using direct-push injections in a grid pattern or by establishing a temporary circulation system to distribute amendments, sometimes referred to as biozones. Generally, more closely spaced wells will increase drilling costs but reduce the duration of an injection event. Conversely, wells spaced farther apart will decrease drilling costs but increase the duration of an injection event. It is important to note that spacing the wells too far apart can make the remediation ineffective, so care must be taken to understand the factors affecting the radius of influence of injection wells. Selecting the most cost-effective grid spacing is evaluated on a site-by-site basis, and is dependent on drilling costs, amendment delivery implementation costs, and local site characterization.

The following general points should be considered when planning well spacing:

- Closer well spacing is needed at sites with low-permeability soils.

- 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.
- For the areas at a site that have especially high contaminant concentrations, closer well spacing is often appropriate to enhance delivery of amendments to those areas where the demand is the greatest.
- Depending on the soil types present, direct delivery of amendments into the contaminated material using closer well spacings can deliver amendments more quickly than relying on groundwater advection/dispersion and could decrease the treatment timeframe.

Well Orientation. Both horizontal and vertical wells can be used to treat subsurface petroleum releases with an EAOB system. 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, such as a building, or if the thickness of the saturated zone is less than 10 feet. Horizontal wells are generally only an effective delivery mechanism in sites with relatively stable groundwater elevations and over short well lengths, under 100 feet. Long horizontal injection wells may require custom screen designs to vary the open area of the slots along the length of the well in order to prevent short-circuiting of injectants; more open area may be needed at the terminal end of the well than closest to the source of the injectants to ensure amendments are not disproportionately displaced near the entrance to the well.

Well Construction. In-situ bioremediation system wells are generally constructed of one- to six-inch diameter PVC, galvanized steel, or stainless steel pipe. At sites where sulfate amendments are planned, it may be advisable to avoid using galvanized steel or stainless steel, because sulfide is highly corrosive. Injection wells typically have screen lengths that extend from the base of the wells into the unsaturated zone. Extraction wells should be screened in the saturated interval containing the greatest mass of hydrocarbons. Field-scale pilot studies, subsequent data analysis, and hydraulic modeling can help to determine the configuration and construction design of groundwater extraction and injection wells.

Implementation costs increase with greater depth and treatment thickness. When injection wells are used, initial injection rates are typically similar to the theoretical transmittance capacity of the screens but can be adjusted in the field to ensure distribution across the entire screened interval. Careful consideration for injection well screens is important. Large-diameter wells with high-flow screens may seem to be the best approach to inject fluids, but this approach may not result in effective distribution of amendments. For example, if a selected screen has a theoretical transmittance capacity of 2 gallons per minute (gpm) per foot and its total length is 10 feet, the overall theoretical transmittance capacity is 20 gpm. If the designed injection rate on that screen is only 5 gpm, there is a possibility that the screen will not be fully pressurized and injection materials will enter through the top 2.5 feet of screen or the most permeable interval. Well diameters and screen characteristics, such as the size and type of openings, need to be specifically designed with the aquifer material and target injection rate in mind. Once installed, thorough well development is required to maximize injection efficiency. Before amendments are applied to a site, the injection wells must be evaluated to assure they can still operate at their designed injection rates.

In a single injection point, multiple screens of shorter lengths – perhaps 5 to 10 feet long – may be required to achieve adequate vertical distribution, rather than one long screen. Injection wells intended to provide multiple injection depths are commonly installed as well clusters, sometimes bundled in the same borehole or as individual wells in separate boreholes but closely spaced together. The optimal injection well design will be site-specific. The well cluster approach may have higher associated drilling costs but result in lower injection costs because downhole packers or other equipment are not needed.

Nutrient Concentrations

The rate of degradation is typically limited by the availability of electron acceptors; however, nutrient amendments may be necessary as well. Microbial populations need various nutrients to sustain their growth, including the major nutrients nitrogen, phosphorous, potassium, and sulfur, and the minor nutrients magnesium, calcium, manganese, iron, zinc, copper, sodium, and trace elements. Although microbial activity could decrease if nutrients are not available in sufficient amounts, nutrient deficiencies are typically not the growth-limiting factor when poor performance occurs. In fact, many practitioners of in-situ bioremediation do not add any nutrients beyond those that are naturally occurring. Anaerobic systems do not need nutrient concentrations as high as the concentrations required by aerobic systems, because anaerobic microorganisms have lower growth rates than aerobic microorganisms. Baseline characterization of an aquifer can help identify potential nutrient needs, if any. If necessary, nitrogen and phosphorous are usually added to the bioremediation system in a useable form, such as aqueous solutions containing ammonium and phosphate ions, for nitrogen and phosphorous, respectively. However, nutrients can cause soil plugging by reacting with minerals, such as iron and calcium, to form stable precipitates that fill the pores in the soil and aquifer.

In some states, groundwater antidegradation policies may limit or prohibit nutrient addition to aquifers without a permit or restrict the use of certain compounds or product formulations. In some localities, there may also be groundwater action levels or cleanup levels for nutrients that you may also need to consider.

Aquifer Matrix Diffusion Potential

Sedimentary aquifers commonly consist of heterogeneous layers or zones of different permeability and transmissivity. Groundwater flows preferentially through more permeable zones as compared with the less permeable zones. Bioremediation is more effective in the more permeable zones because amendments infiltrate much more quickly through these high permeability zones. Contamination often exists in the subsurface for many years before it is detected and remediated. This delay allows the dissolved contaminants the time needed to diffuse from more permeable into less permeable zones within the aquifer system. When remediation begins, the high permeability zones are remediated more quickly and the concentration gradient between high and low permeability zones is reversed. As a result, contaminants in the less permeable matrix diffuse back into the more permeable matrix, causing contaminant levels in the more porous matrix to rebound after initial treatment. The less permeable matrix of the aquifer becomes a new source area for contamination. This matrix back-diffusion, also generically called "rebound," may persist for many years after initial treatment.

The slow release of contaminant mass from matrix back-diffusion can hinder attainment of cleanup level goals. Matrix back-diffusion can be significant where low-permeability zones are present within the unconsolidated aquifer or where a dual porosity system exists as a function of adjacent lithologic units having several orders of magnitude differences in hydraulic conductivity. Matrix back-diffusion can also play a significant role in bedrock aquifers that exhibit sufficient primary porosity. For example, numerical model simulations have demonstrated that back-diffusion from the matrix pore space, primary porosity, to fractures, secondary porosity, will likely be the time-limiting factor in reaching groundwater cleanup goals in some fractured bedrock environments (Lipson 2005). Matrix back-diffusion occurs much more in sedimentary rocks than in igneous and metamorphic rocks.

Additional applications of amendments may be required to maintain a biologically active zone that will ultimately reduce contamination to below the remedial action objectives. In some cases, several pore volumes of treated groundwater may have to pass through the aquifer before objectives are met. Remediation of aquifers where matrix back-diffusion is a factor may take longer and be more costly. Research is under way to develop ways to estimate the rate of diffusion from the matrix into the groundwater (EPA 2013).

Evaluation Of Operation And Monitoring Plans

Remedial Performance Monitoring

Significant uncertainties associated with site conditions can remain even as remedial designs are completed and implemented and may result in remedial operations that vary from the design. These variances often require adjustments to optimize system performance. Evaluation sampling is performed to gauge the effectiveness of the EAOB system relative 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 can be made to optimize system performance early in the remediation program. Projects with regular performance reviews have a greater chance of achieving the design remedial goals within the projected time and cost estimates.

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

- Delivering electron acceptors and any other amendments to the saturated zone at required design rates
- Distributing electron acceptors across the target contaminated area
- Reducing concentrations of petroleum hydrocarbons in soil and groundwater at design rates through biodegradation of the petroleum compounds

For any cleanup, it is important to measure progress toward the remedial objectives, in order to optimize the remedy and meet the objectives. Depending on the remedial objectives, it may take a few years or decades to reach remedial objectives. Key questions to ask when a monitoring approach is developed include:[15]

- What media should be monitored?
  - Is soil vapor intrusion a concern?
  - Do sub-slab vapors or indoor air quality need to be considered?
  - Are there any adjacent surface water bodies that need to be monitored in addition to the main remediation area soil and groundwater?

- What constituents should be monitored?
  - Beyond the contaminants of concern (COCs), what other parameters should be monitored to establish multiple lines of evidence to evaluate performance?
  - How many lines of evidence are needed for an assessment toward an objective?

- What metrics should be used?

- Where and how deep should monitoring points be located?

- When and how often should monitoring occur?
  - Do seasonal issues or tidal issues need to be considered?

- What is the timeframe for use of the groundwater resource?

Exhibit XIV-22 identifies the parameters that can be measured in groundwater, soil, and soil vapor samples to evaluate EAOB progress and system performance. We also provide a brief description of the respective sampling frequencies, and the relevance and significance of each parameter to the performance evaluation. With biologically dependent remedies, the initial monitoring is critical to the overall success of the remedy to ensure aquifer geochemistry has responded as bench and pilot testing suggested; target bacterial communities are established, sustained, and thriving; and to track the general biogeochemical responses to the initial installation. For example, initial performance monitoring could be monthly for a quarter, followed by quarterly for a year and semi-annually thereafter. It is important to evaluate performance data after each injection event to ensure that expected trends are observed. Even at sites where initial performance is promising, the groundwater monitoring program must consider the possibility of rebound caused by various factors, including matrix back-diffusion.

A key element is the locations where performance evaluation sampling takes place relative to subsurface amendment delivery points. Performance evaluation samples should not be collected from

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[15] ITRC 2011

amendment delivery locations**.** The performance of the EAOB system should be determined by the chemistry and microbiology of soil and groundwater located between, around, and downgradient of amendment delivery locations rather than inside or in the immediate vicinity of the amendment delivery points. Conditions inside or in the immediate vicinity of injection locations have been preferentially altered by EAOB 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 EAOB system is having on the subsurface conditions as a measure of its performance, samples of soil, groundwater, and soil vapor should be collected from alternate locations.

A performance monitoring program is intended to measure performance at key locations within a plume. In general, wells would be located within the biologically active area, immediately downgradient of the biologically active area, and farther downgradient at distances based on site seepage velocities, monitoring frequency, and any regulatory requirements. In reviewing the performance monitoring plan in the CAP, you should verify that a sufficient number of sampling locations exist between amendment application points to provide the necessary performance sampling data.

As petroleum hydrocarbons biodegrade, they break down into other compounds, called degradation daughter constituents. As stated in Exhibit XIV-22, the monitoring program can use the concentrations of these daughter constituents to determine whether biodegradation is happening. Exhibit XIV-23 provides examples of some daughter constituents that can be used to monitor anaerobic bioremediation.

Exhibit XIV-22
Common Performance Monitoring Parameters And Sampling Frequencies

Analytical Parameter	**Sampling Frequency**			**Purpose**
	Startup Phase (first month)	**Remediation/ Post-Application Long-Term Monitoring Phase (on-going)**		
	Daily	***Weekly To Monthly***	***Quarterly To Annually***	
Groundwater				
Electron acceptor	X	X		• Determines system's effectiveness in distributing electron acceptor in treatment area • Provides data to optimize system performance
Redox potential	X	X		• Provides indication of reducing or oxidizing conditions in the subsurface with changes over time used to help evaluate effectiveness of amendment additions
pH	X	X		• Confirms pH conditions are stable and suitable for microbial bioremediation • Identifies trends of concern
Nutrients (if being used)	X		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
Degradation daughter constituents			X	• Offers direct evidence of contaminant bioremediation and enhanced anaerobic • bioremediation effectiveness
Water table elevations	X	X		• Determines if hydraulic conditions (groundwater flow) are consistent with design intent • Determines if project has had an unanticipated effect on these conditions
Byproducts (e.g., metals, TBA increase, hydrogen sulfide)			X	• Determines whether the bioremediation is causing unacceptable levels of harmful byproducts

Exhibit XIV-22
Common Performance Monitoring Parameters And Sampling Frequencies

Analytical Parameter	Sampling Frequency			Purpose
	Startup Phase (first month)	Remediation/ Post-Application Long-Term Monitoring Phase (on-going)		
	Daily	*Weekly To Monthly*	*Quarterly To Annually*	
Dissolved oxygen	X	X		• Helps with troubleshooting
Soil Vapor				
Carbon dioxide	X	X		• Provides evidence of potential biodegradation
Volatile petroleum COCs	X	X		• Suggests residual sources in soil or fugitive emissions associated with the remedial effort
Soil				
Petroleum COCs			X	• Provides a measure of remedial progress and the extent to which biodegradation of sorbed contaminants is limited by the presence of source material

Exhibit XIV-23
Degradation Daughter Constituents Used To Monitor Anaerobic Bioremediation

Target Contaminant	Example Daughter Constituents
Benzene	Phenol (Suthersan et al. 2011)
Toluene	Cresol (Suthersan et al. 2011) Benzylsuccinate (Chakraborty and Coates 2004) (Benzylsuccinic acid (Farhadian et al. 2008)) e-phenylitaconate (Chakraborty and Coates 2004)
Xylene	Methylbenzylsuccinate (Chakraborty and Coates 2004) (Methylbenzylsuccinic acid (Farhadian et al. 2008))

Another strategy for monitoring the progress of in-situ biodegradation is stable-isotope analysis. This strategy takes advantage of the fact that a chemical element can have several versions with different masses, depending on how many neutrons are in the atom; these different weight versions are called isotopes. Different samples of the same compound can have different isotopic signatures depending on the proportions of the various isotopes they contain. Researchers can study a sample's isotope ratio to learn about the origin of the sample and whether that sample has undergone biodegradation.

Isotope analyses usually use carbon but can also involve hydrogen (EPA 2013). One type of isotope analysis, called compound-specific isotope analysis, measures the changes in a contaminant's isotope ratio to determine whether biodegradation is occurring. Because microbes can more easily break the bonds of light isotopes, the degradation rate for light isotopes is greater (ITRC 2013). Therefore, as biodegradation proceeds, the remaining contamination will contain an increasing proportion of heavy isotopes.

Stable isotope probing is another isotope-based tool. Stable isotope probing artificially enriches a sample of a contaminant with high levels of a stable isotope. This labeled sample is then introduced to the environment. Researchers are able to follow the labeled contaminant as it disperses through the environment, allowing them to determine whether biodegradation is happening (EPA 2008, ITRC 2013, Kolhatkar et al. 2002, Kuder et al. 2005, Wilson et al. 2005b).[16] See ITRC 2013 for more information about compound-specific isotope analysis and stable isotope probing.

Maintenance Of The Remedy

The expected outcomes vary for each bioremediation strategy, but generally include reductions in contaminant concentrations, stable redox conditions, stable geochemistry, and adequate amendment concentration. However, a short-term increase in contaminant mass may occur initially after installation of the remedy because of changes in the equilibrium between the contaminant phases (adsorbed, dissolved, and NAPL) and transition of contaminant into the dissolved phase due to the formation of bio-surfactants. The following conditions may indicate poor performance of a bioremediation remedy:

- Limited, incomplete, or no reduction, and even increases in contaminant concentrations (However, temporary increases in contaminant concentrations can be expected near source zones because of increased dissolution and may be considered a positive sign for possible future contaminant reductions. This is generally evident in the first six months to a year following any injections of amendments. However, if the increase persists for longer timeframes a re-evaluation of the data may be warranted.)
- Trending toward aerobic conditions in a remedy meant to be anaerobic
- Sharply decreasing concentrations of amendments or amendment concentrations below those necessary to support bioremediation at a site

When these conditions occur, modifications in the system may be necessary to improve performance. Possible modifications may include changes in the method of amendment delivery, changes in the selected amendment, and conditioning the aquifer geochemistry.

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[16] See ITRC 2013 for more information about compound-specific isotope analysis and stable isotope probing.

Re-application of amendments, including electron acceptor, will be required at most sites. Some sites may require geochemical adjustment and nutrient amendments. New wells with shorter or focused screens or shorter target injection intervals may be needed in areas where data suggest amendment distribution is inadequate.

The success of biological technologies depends on the presence and persistence of the amendment and maintaining the geochemical conditions in groundwater that will allow biological populations to flourish. The aquifer's pH is critical to the performance of bioremediation systems. Adjustment of pH during subsequent maintenance applications, typically with the addition of a base like sodium bicarbonate or sodium hydroxide may be needed; however, pre-design data should inform a practitioner of the potential need for post-installation pH adjustment. An effective performance monitoring program is required to decide when additional amendments are required to maintain the biologically active zone.

Biofouling is attributed to the increase in microbial populations and, perhaps more importantly, to cells creating extracellular polysaccharides. These slimy polysaccharides contribute to the accumulation of microorganisms on surfaces or within porous media and can contribute significantly to biofouling of injection wells. A portion of the supplied amendment mass goes to the creation of new bacteria biomass. Continued unchecked bacterial growth is likely to eventually reduce circulation and injection of the amendment and may lead to a plugged injection well. Biofouling of injection or recirculation wells has occurred at several sites because of biomass or biofilm growth within the well screen and the surrounding sand pack. Several approaches have been used to mitigate these effects, and biofouling should not be considered a major impediment to EAOB. Measures to prevent biofouling include pulsed injection and injection of biocides to control bacterial growth; fouled wells can be rehabilitated using conventional redevelopment techniques, scrubbing with hydrogen peroxide, and injection of carbon dioxide under pressure (Air Force Center for Environmental Excellence et al. 2004; EPA 2013).

Unintended Consequences

Evaluation sampling should monitor for unintended consequences of anaerobic bioremediation. One in particular is the mobilization of metals that are naturally occurring in the aquifer. Excessive organic loading of an aquifer, such as from a fuel release, will result in biological activity that will readily consume oxygen and drive a system to reducing conditions. The resulting reducing environment may cause metals to become mobile. Arsenic mobilization is of particular concern in areas with naturally occurring arsenic in soils or bedrock. Mobilized metals will persist until the oxidation-reduction state shifts back to oxidative and metals form oxides, making them generally immobile. The shift back to oxidative conditions occurs naturally downgradient of a biological treatment area; however, an engineered approach such as an air-sparge wall could be installed to induce oxidative conditions if receptors are present (EPA 2013).

Other potential unintended consequences related to the reducing conditions may include:

- Nitrate is reduced to nitrite, nitric oxide, nitrous oxide, nitrogen gas, or a combination of these byproducts, depending on the microbes that are present. Nitric oxide, nitrous oxide, and nitrogen gas are gaseous byproducts that can dissolve into groundwater to some extent, but will generally escape into the vadose zone; however, the gaseous byproducts may become trapped within pore spaces, displacing water and reducing the hydraulic conductivity of the saturated matrix.

- Manganese (IV) and iron (III) are reduced to soluble manganese (II) and iron (II), respectively. These dissolved-phase metals may contribute to secondary groundwater plumes and elevated total dissolved solids.
- Sulfate is reduced to sulfite and sulfide. The end-product of sulfate reduction is sulfide. At sites with high sulfate concentrations and not enough dissolved metals to precipitate the sulfide, hydrogen sulfide gas is generated, which is toxic and flammable and could result in vapor intrusion issues depending on the depth of the plume and characteristics of any overlying buildings.
- Fermentation generates hydrogen ions, which can lower the pH of the groundwater to levels where the key bacteria cannot survive. In addition, carbon dioxide is reduced to methane, which can support a community of microbes called methanotrophs, but could result in vapor intrusion issues.

Therefore, you should ensure that the operation and monitoring plan includes plans for monitoring metals, as well as other potential byproducts associated with the proposed electron acceptor.

Evaluation Criteria

Remedial performance monitoring data provide the evidence needed to assess EAOB system performance. This evidence requires examination and interpretation to confirm EAOB system effectiveness and whether system modifications are necessary. Below is a discussion of how these data can be interpreted to evaluate system performance. The discussion focuses on two broad EAOB system requirements:

- Amendment delivery and distribution
- Anaerobic biodegradation

Amendment Delivery and Distribution – Performance sampling may indicate that the EAOB system is meeting design specifications for amendment delivery and distribution if the data show the following:

- The remedy is delivering amendments to the subsurface at the mass delivery rate required by the design.

- The remedy has elevated the concentrations of dissolved electron acceptors in groundwater samples collected across the target treatment area.

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.

Anaerobic Biodegradation – If performance monitoring shows that the following criteria are met, then the EAOB system is effectively causing biodegradation:

- Decreasing dissolved and sorbed petroleum contaminant concentrations, or a 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
- Production of carbon dioxide in the saturated zone as evidenced by total inorganic carbon in the groundwater
- Significantly increased microbial activity in the contaminated area as suggested by comparison of baseline and subsequent microbial population quantification
- Isotope analysis shows that biodegradation is occurring

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 EAOB system is effective. For example, apparent contaminant reductions in dissolved and sorbed phases could occur because of groundwater advection and dispersion or simply because of natural fluctuations in water levels. Alternatively, if hydraulic manipulation, such as engineered hydraulic gradients, of the groundwater is part of the bioremediation system, apparent contaminant reductions could result from dilution or separation of the groundwater from the contaminated soil, for example, if the water table is depressed below the contamination. In this case, contamination levels in groundwater could rebound to near preexisting concentrations if you turn off the hydraulic controls and groundwater re-contacts the contaminated soil.

The most direct indication that EAOB has caused increased numbers of hydrocarbon-degrading bacteria is observation of significantly increased populations of hydrocarbon-degrading bacteria in the target treatment area. Surveys of genes associated with hydrocarbon-degradation are a way to document an increase in a degrading population. In addition, if performance sample analyses detect intermediate degradation daughter products, this may be further evidence of contaminant biodegradation that has been enhanced.

Overall, densities of targeted bacterial populations should increase with time and reach optimal levels (>10^7 cells/L), geochemical conditions must remain favorable, and contaminant levels should decrease in all performance monitoring wells and eventually in downgradient point of compliance wells for successful bioremediation applications. If progress toward remedial objectives is not adequate, re-evaluation of a remedy could be warranted (EPA 2013).

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Checklist: Can Enhanced Anaerobic Oxidative Bioremediation Be Used At This Site?

This checklist can help to evaluate the completeness of the CAP and to identify areas that require scrutiny. In reviewing the CAP, answer the following questions. If the answer to several questions is "no," request additional information to determine if the proposed EAOB technology and approach will effectively accomplish the site cleanup goals within a reasonable time. Note that many of the items included in this checklist also apply to other in-situ bioremediation methods, in addition to EAOB.

1. Site Factors

Yes	No	
○	○	Is the soil hydraulic conductivity greater than 10^{-4} cm/sec?
○	○	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?
○	○	Does the soil profile of the contaminated zone contain high levels of natural organic material (e.g., layers of peat or humic material)?
○	○	If the groundwater pH is outside the neutral range (6-8), has the CAP determined whether pH adjustment is needed?
○	○	Is groundwater temperature between 10°C and 45°C?
○	○	Has the CAP established that anaerobic or facultative hydrocarbon-degrading microbes are present?
○	○	Has the CAP established that the site's contaminants are sufficiently biodegradable?
○	○	Are the site's contaminants at least slightly soluble in water?
○	○	Have imminent likely excessive risks to human health or the environment (if any, associated with the petroleum contamination) been eliminated?
○	○	Have other techniques been used to address any NAPL or extremely high dissolved or adsorbed phase contaminant concentrations?
○	○	Will the state allow the proposed bioremediation system to be implemented without permits?
○	○	Has the CAP determined whether the type and concentration of electron acceptor proposed for use will adversely affect drinking water aquifers; exceed federal, state, or local standards; or violate state groundwater antidegradation rules?
○	○	Is the depth to groundwater greater than 5 feet (to prevent daylighting during amendment injections)?
○	○	Has the CAP determined that any utilities within the treatment area would not be adversely impacted by the amendments being added?
○	○	Has the CAP determined whether hydraulic control is needed to prevent off-site migration of contaminants?

2. Enhanced Anaerobic Oxidative Bioremediation Design

Yes	No	
○	○	Has the mass of petroleum hydrocarbons requiring biodegradation been estimated?
○	○	Has the mass of electron acceptor required to biodegrade the petroleum contaminants been estimated?

○	○	Can the proposed EAOB approach deliver the necessary electron acceptor mass to the treatment area within the estimated cleanup time?
○	○	Is the capacity of the EAOB treatment system sufficient to deliver electron acceptors at the required design rate?
○	○	Is the density and configuration of injection wells adequate to uniformly disperse dissolved electron acceptors through the target treatment zone, given site geology and hydrologic conditions?
○	○	If the CAP proposes to use a recirculation system, does the CAP ensure that the extraction wells will capture the added groundwater gradient created by the injection wells?

3. Written Performance Monitoring Plan

Yes	**No**	
○	○	Will a comprehensive set of baseline sampling be performed prior to EAOB system start-up?
○	○	Does the plan specifically exclude sampling from injection wells when collecting final performance evaluation data?
○	○	Are monitoring wells adequately distributed between the amendment delivery locations?
○	○	Does the written plan include periodically collecting soil samples from the contaminated intervals at locations between amendment delivery locations?
○	○	Will the soil, soil vapor, and groundwater samples be analyzed for the recommended performance monitoring parameters?
○	○	Will frequencies of performance monitoring generally correspond to those identified in Exhibit XIV-22?