Appendix A: Amendments and Other Additives

The following Fact Sheets discuss the Amendment and four topics related to it:

- Limitations
- Other Considerations
- Health and Safety
- Additional Links and Information

It is important to recognize that many amendments blur the lines between biotic and abiotic applications. Amendments are grouped under biotic and abiotic amendments in Sections A1 and A2, respectively, below based upon what we believe are the primary applications, but recognize that many amendments may fit into more than one category considered in multiple sections. Other additives, such as nutrients, pH modifiers, and methane inhibitors, are summarized in Section A3. Care should be taken to evaluate the likelihood of effective distribution of the amendment within the desired treatment zone, and of the effective microbial use of the amendment for beneficial transformation of the contaminant to meet the treatment goals. Care should also be taken to avoid adverse effects on the compliance monitoring wells. The current state of the practice often allows practitioners to be successful with any of these amendments given proper evaluation, planning, and application. Details of typical delivery methods are discussed in Amendment Delivery Optimization Section 3.6.

A1 Common Biotic Amendments

This section describes biotic amendments that are used to enhance biological degradation processes. This includes products designed to stimulate aerobic or anaerobic metabolic processes, and also cometabolic biological processes. Collectively, these three categories are intended to create optimal conditions for naturally occurring bacteria. The bacteria may use a variety of electron acceptors (oxygen, nitrate, sulfate, manganese, iron, carbon dioxide, yielding methane and chlorinated solvents). Growth of bacteria is favored at neutral pH, moderate temperatures, and the presence of inorganic nutrients such as nitrogen, phosphorus, and potassium. In addition, specialized bacteria can be added in a process called bioaugmentation, which is discussed in Section A1.4.

A1.1 Aerobic Bioremediation

In the presence of aerobic conditions and appropriate nutrients, microorganisms can convert many organic contaminants to carbon dioxide, water, and microbial cell mass. Many organisms are capable of degrading hydrocarbons using oxygen as the electron acceptor and the hydrocarbons as carbon and energy sources. Aerobic metabolism is more commonly employed and can be effective for hydrocarbons and other organic compounds such as petroleum hydrocarbons and some fuel oxygenates (for example, methyl tertiary-butyl ether [MTBE]). Aerobic bioremediation technologies may also change the ionic form of metals, though the permanence of these changes will depend on site-specific conditions. If a site contains mixed metal and organic wastes, it is necessary to consider whether the oxidized forms of the metal species (such as arsenic) will be environmentally acceptable (USEPA 2006b).

Aerobic oxidation can occur naturally under proper conditions, but oxygen, which is often considered to be the primary growth-limiting factor for hydrocarbon-degrading bacteria, is normally depleted in zones that have been contaminated with hydrocarbons. Common amendments used for aerobic bioremediation are air, pure oxygen, hydrogen peroxide, ozone, and commercial oxygen-releasing compounds such as magnesium peroxide, calcium peroxide, and calcium oxy-hydroxide. More information about the common amendments used for aerobic bioremediation is available in Table 3-2. Enhanced aerobic bioremediation technologies focus in part on increasing oxygen levels and can potentially increase biodegradation by several orders of magnitude over naturally occurring, nonstimulated rates. Enhancements can be used to address contaminants in the unsaturated zone, the saturated zone, or both. The stoichiometric ratio of oxygen per hydrocarbon is about 3 moles O_2 per 1 mole of hydrocarbons. The success of aerobic bioremediation highly depends on the ability to deliver oxygen to the hydrocarbon-degrading microorganisms. The effectiveness of a bioremediation system is largely dictated by the balance between oxygen sources, the oxygen uptake, and the degree to which oxygen is transported through the subsurface (USEPA 2004).

Technologies to accelerate in situ aerobic bioremediation include biosparging, bioventing, and directly injecting oxygen-releasing substances. These technologies work by providing an additional supply of oxygen to the subsurface, which then becomes available to aerobic bacteria. Most enhanced aerobic bioremediation technologies primarily address contaminants that are dissolved in groundwater or that are adsorbed to soil particles in the saturated zone. Enhanced aerobic bioremediation technologies are typically used outside source areas (USEPA 2004). To enhance aerobic bioremediation, nutrients (nitrogen, phosphorus, magnesium, etc.) and pH buffers may be added to the groundwater. More detailed information about the additives (nutrients, pH buffer, etc.) can be found in Section A3.

Limitations

- Fouling or clogging of the aquifer may occur due to the precipitation of oxygenated metal species, particularly iron and manganese.
- Biofouling may also occur when bacteria attach to, grow on, and block the well screen, filter pack, or formation surrounding a nutrient delivery well. Nitrate- and perchlorate-reducing bacteria have been found to cause significant fouling of nutrient delivery systems.
- Certain metals may be mobilized in the subsurface. Care must be taken to protect receptors such as surface water from mobilized metals plumes.
- Strong sources, including NAPL, are generally not conducive to aerobic biodegradation.

Other Considerations

- Electron acceptors are required for aerobic reactions.
- Ambient subsurface conditions (intrinsic permeability, groundwater gradient, conductivity, etc.) should be studied to ensure that they are conducive to placement and distribution of amendments and the natural geochemistry (pH, dissolved oxygen, ORP, dissolved iron concentration, etc.) is appropriate for aerobic reaction.
- Certain amendments may have patent restrictions.
- Some restrictions may apply due to concentrations of constituents in the amendment—that is, salts, metals.

Health and Safety

- General drilling considerations are required.
- Precautions are required when dealing with oxygen tanks, etc.

Additional Information

More detailed description of common amendments used for aerobic bioremediation can be found in (ESTCP 2005a; ITRC 2008b; USEPA 2000).

A1.2 Cometabolic Aerobic and Anaerobic Bioremediation

Cometabolic bioremediation may be aerobic or anaerobic. In either form, the contaminant is degraded as the result of microbial metabolism of another compound. The biodegradation of the contaminant does not yield any energy or growth benefit for the microbe mediating the reaction (USEPA 2000). In aerobic cometabolic bioremediation, the contaminant is oxidized (loses an electron) by an enzyme or co-factor (a substance, other than the substrate, whose presence is essential for the activity of an enzyme) produced during microbial metabolism of another compound with oxygen. In anaerobic cometabolic bioremediation, the contaminant is reduced (gains an electron) by an enzyme or co-factor produced during microbial metabolism of another compound in an environment devoid of oxygen.

Cometabolic processes used for the bioremediation of COCs involve increasing the populations of organisms that generally exist and propagate by consumption of a primary substrate (for example, methane or propane) while producing enzymes that fortuitously degrade the COCs. Because the organisms obtain no benefit from the ancillary degradation of the COCs they can persist and thrive in the absence of the COCs. As an example; the bacteria *Pseudomonas methanica* (a methane-oxidizing, or "methanotrophic" organism) degrades its primary growth substrate, methane, by production of the enzyme methane monooxygenase, which will then degrade many COCs even though the bacteria obtain neither energy nor carbon from these ancillary degradation reactions.

Although both aerobic and anaerobic cometabolic biodegradation of COCs has been observed, most cometabolic remediation applications are aerobic. COCs that have been shown to be degraded cometabolically under aerobic conditions include TCE, cis-DCE, VC, TCA, chloroethane, chloroform, methylene chloride, MTBE, 1,4-dioxane, THF, TNT, RDX, atrazine, PAHs, and some pesticides. For example; the general aerobic cometabolic biodegradation pathway of trichloroethene (TCE) by methanotrophs is shown in Figure A1-1 below.
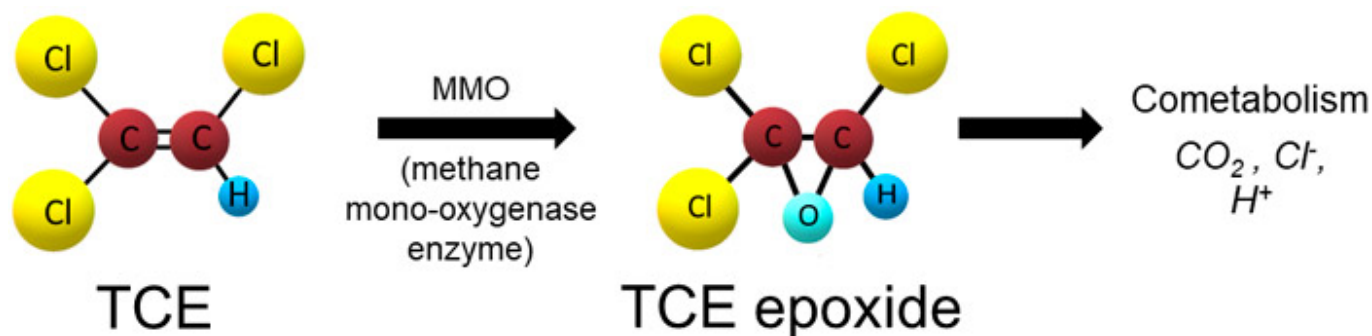
Figure A1-1. TCE oxidation pathways in methane-oxidizing bacteria.

The most common primary substrate amendments for aerobic cometabolic bioremediation include light alkanes, such as methane, propane, and butane, and alkenes, such as ethene and isobutene. If already present at the site in groundwater, bacteria can use other compounds such as toluene, phenol, methanol (Little 1988), and ammonia by as primary substrates.

Nutrients used to enhance cometabolic microbial growth include nitrogen, phosphorus, iron, magnesium, calcium, and trace elements (zinc, manganese, boron, cobalt, copper, nickel, and molybdenum). Oxygen is added to the subsurface to maintain sufficient aerobic conditions, and pH buffers such as sodium bicarbonate are added, as needed, to establish and sustain appropriate groundwater pH levels for the bacteria population. More detailed information about the additives (nutrients, pH buffer, etc.) can be found in Section A3. More detailed information about the bioaugmentation cultures can be found in Section A1.4.

Limitations

- Cometabolic bioremediation is limited by lists of contaminants that can be successfully cometabolized. For example, PCE and CT are not thought to be cometabolically biodegraded.
- Favorable subsurface geochemical conditions are required.
- Certain intermediates from the process may be toxic to microorganisms producing the enzymes.
- Under aerobic conditions, primary substrate and oxygen levels must be balanced to grow enough cells without oxygen depletion.

Other Considerations

- A contaminant itself may be used as a substrate in certain instances.
- Nutrients, oxygen, and pH buffers are added as needed to enhance microbial growth and maintain desirable subsurface conditions.
- Certain amendments may have patent restrictions or regulatory limitations based on ancillary components such as salts and metals.
- Bioaugmentation may be beneficial in certain cases.

Health and Safety

- Some gaseous substrates may be flammable.
- Hazards associated with substrates, nutrients, oxygen, and organisms must be taken into consideration.

Additional Information

- Bioremediation Review USEPA Cleanup Information (Last updated on February 7, 2019).
- (USEPA 2000). Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications.

A1.3 Anaerobic Biological Reduction

Under anaerobic conditions, certain types of microbes are capable of deriving energy by respiring organic compounds resulting in degradation of the organic compound via a reductive process. In general, anaerobic conditions are used to degrade highly halogenated contaminants though some petroleum hydrocarbons may also be biodegraded anaerobically. The halogenated compound, typically a chlorinated solvent, serves as the electron acceptor while hydrogen serves as the direct electron donor (USEPA 2000). Bacterial respiration results in replacement of a halogen (such as chlorine) with hydrogen, resulting in reduction of the parent compound. In bioremediation, the desired objective is to completely reduce contaminants to nonhazardous and environmentally acceptable products such as ethene, ethane, carbon dioxide, and

chloride. Other bacteria can reduce inorganic compounds such as certain metals. Information regarding microorganisms is provided in Section A1.4.

Since the process of bioremediation was recognized in the early 1980s, efforts to enhance these biological processes have focused on development and distribution of organic substrates that can be effectively fermented to produce the hydrogen needed for dechlorination. Many types of electron donor amendments are used to promote reductive dechlorination (see "In Situ Bioremediation and Soil Vapor Extraction at the Former Beaches Laundry & Cleaners"). Examples include carbohydrates such as molasses, corn syrup and alcohols; carboxylic acids and triacylglycerols such as vegetable oils (that is, soybean, canola, etc.); and complex organics such as food processing byproducts (cheese whey) and natural organic matter (mulch) (see "In Situ Biological and Chemical Reduction of Hexavalent Chromium and Perchlorate"). Although electron donors can be categorized according to their chemistry, another way to characterize electron donors is according to their solubility. This allows a better understanding of their injectability. In general, these and other substrates can be divided into major categories based on the solubility properties of the amendment, for example, readily miscible and slowly soluble. The main differences in these categories are the injectability (Section 3.7) mechanisms by which the amendment can be effectively distributed within the treatment area (readily miscible) and the period that each amendment can be expected to remain reactive to promote the desired microbial processes (increases solubility). These characteristics are a function of the physical nature and properties of each amendment (ESTCP 2010a).

Limitations

- Anaerobic biological reduction is relatively slower than abiotic processes such as ZVI or ISCO.
- Degradation products may pose a greater risk than the parent product in some instances (for example, VC).
- Site-specific variables may inhibit establishment of appropriate conditions required for anaerobic remediation.
- Anaerobic conditions generated during remediation may affect other water quality characteristics of a site such as mobilization of iron and manganese or formation of methane.

Other Considerations

- Distribution of the amendment within the desired treatment zone and effective microbial use of the substrate should be evaluated.
- Solubility of the substrate should be considered to understand injectability and distribution in the subsurface.
- Certain amendments may have patent restrictions or regulatory limitations due to ancillary components of the amendments, such as salt and metals.
- Bioaugmentation may be necessary in some cases where there are low numbers of *Dehalococcoides* population and native microorganisms can't biodegrade all constituents (for example, VC).

Health and Safety

- Pressurized line hazards may be present.
- Proper storage and handling of amendments must be considered.
- This method may involve handling of pressurized and heated materials.
- Certain substrates may be considered hazardous due to flammability, corrosivity, etc.

Additional Information

- (ESTCP 2005a) Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs.
- (USEPA 2000) Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications.
- (Government of Canada 2019) Bioaugmentation Fact Sheet
- (NAVFAC 2018) Advances in the State of the Practice for Enhanced In Situ Bioremediation 2018

A1.4 Bioaugmentation

Bioaugmentation is the process of adding microorganisms to the subsurface to enhance the existing microbial population and further promote the biodegradation of contaminants in the soil and/or groundwater. The selected microorganisms may be cultivated from existing populations present at a site (that is, indigenous) and grown in a laboratory, or from specially cultivated strains of bacteria having known capabilities to degrade specific contaminants (that is, nonindigenous). Bioaugmentation can be used to degrade contaminants in either aerobic or anaerobic conditions.

To effectively implement bioaugmentation, it is important to identify existing populations of indigenous microorganisms

suitable for biodegradation and to evaluate:

- the functional genes of the organisms that tell us whether the contaminant can be degraded by that population
- their nutrient requirements
- the appropriate methods for stimulating degradation of target contaminants while minimizing competitive or undesirable microbial activities

The following is a list of the most common microorganisms typically used during anaerobic bioaugmentation:

Dehalococcoides is the most common microorganism genus used for anaerobic bioaugmentation. Only microorganisms belonging to the genus _Dehalococcoides_ and _Dehalogenimonas_ have demonstrated the capacity to dechlorinate dichloroethenes and vinyl chloride to ethene. _Dehalococcoides_ also dechlorinate chlorobenzenes, polychlorinated dibenzodioxins, and PCBs. _Dehalococcoides_ are microorganisms that require anaerobic conditions. Anaerobic biodegradation of target contaminants is often enhanced by an inoculation with _Dehalococcoides_ or other appropriate microorganism-containing culture. Some strains of _Dehalococcoides_ can metabolically degrade VC while others lack the enzymes for direct use of VC and may cometabolically biodegrade VC (this process is often slower and may require parent compounds). Therefore, it is important to know if the indigenous microbial population expresses the vcrA or TCA functional genes for VC reduction. Other VC reductase genes may occur but have not yet been identified.

Dehalobacter restrictus are bacteria capable of reductive dechlorination of chlorinated ethenes and chlorinated ethanes and fermentative dehalogenation of dichloromethane.

Sulfur-reducing bacteria use reduced or oxidized sulfur compounds in their energy transformations. For example, reduced sulfur compounds, such as sulfide and elemental sulfur, can be used by sulfur-reducing bacteria as electron donors or as energy and electron sources. In contrast, oxidized sulfur compounds, such as sulfate or hydrogen sulfide, can be used by sulfate-reducing bacteria as electron acceptors for the oxidation of organic compounds or matter. Sulfur-reducing bacteria also oxidize organic compounds or matter to obtain energy, but they use zero-valent sulfur as an electron acceptor.

Methanogens are microorganisms that produce methane as a metabolic byproduct in anoxic conditions. It is desirable and typical for _Dehalococcoides_ to dominate the microbial population when promoting the dechlorination of dichloroethenes to ethene. However, in highly reductive environments, methanogens may dominate. Methane inhibitors may be introduced to manage and/or reduce methane concentrations during bioremediation. More detailed information regarding methane inhibitors can be found in Section A3.3 below.

Microbial consortia consist of two or more kinds of microorganisms acting symbiotically. Consortia provide a varied population of organisms to enhance the biodegradation of a suite of COCs and/or more complete biodegradation. In some cases, one group of microbes may produce co-factors such as vitamin B_{12} that dechlorinating bacteria need to complete the dechlorination reaction or to reduce intermediates that may interfere with further metabolism.

The effectiveness of enhanced aerobic bioremediation is a function of the presence of heterotrophic bacteria in the subsurface. If the background heterotrophic bacteria levels are higher than 1,000 colony forming units (CFU)/gram dry soil, enhanced aerobic bioremediation is generally effective. If the background heterotrophic bacteria levels are less than 1,000 CFU/gram dry soil enhanced aerobic bioremediation may be effective; however, further evaluation is needed to determine if toxic conditions are present (USEPA, 2017).

The chlorinated ethenes can degrade via cometabolic dechlorination (Fathepure 1987), although it is generally held that PCE is not amenable to aerobic cometabolic degradation, despite documentation of aerobic PCE cometabolism by _Pseudomonas putida OX1_ by (Ryoo 2000). Other organisms, including _Pseudomonas putida_ and _Methylosinus trichosporium OB3b_, degrade additional chlorinated compounds (for example, chloroethenes, chloroethanes, chloromethanes, and chloropropanes) via cometabolism (Heald 1994; Oldenhuis 1989). Unfortunately, despite the promise of the aerobic cometabolism approach, field implementation was found to be very challenging and was met with a series of incremental setbacks. First, introducing enough oxygen and cosubstrate proved to be difficult and/or expensive to implement (Steffan 1999). This led to the development of cultures using selected or genetically engineered microorganisms that would constitutively (that is, would not require induction by a metabolite) express these enzymes (Munakata-Marr 1996b). Although genetically engineered microorganisms have been developed, there are very few instances where they have been applied for bioremediation in field settings due to regulatory concerns and survival of the engineered microorganisms (Sayler 2000). However, adhesion of the introduced bacteria in the zone immediately surrounding the injection point limited the distribution of the microorganisms and the success of the bioremediation process for field-scale applications. To overcome this technical hurdle, both adhesion-deficient strains and ultramicrobacteria were developed (Cusack 1992; DeFlaun 1999) that possessed the desired degradation capabilities. However, after many years of field trials, aerobic cometabolism was determined to be too difficult for many to implement and sustain at most sites (relative to enhancing reductive dechlorination), and the approach has generally fallen out of favor for the remediation of chlorinated solvents. Cometabolic dechlorination may be suited for sites

with very low concentrations of chlorinated solvents.

In some cases bioaugmentation with a known cometabolic degrader such as *Burkholderia cepacia G4*, *Methylosinus trichosporium OB3b*, and ENV425, or other propane oxidizers may be beneficial if the required bacteria population is not present or is found at very low numbers ([Munakata-Marr 1996a](#)) ([Chang 1996](#)).

Limitations

- Bioaugmentation culture will need electron acceptors or electron donors and neutral pH to survive.
- Presence of oxygen can significantly impact efficiency of strictly anaerobic microorganisms.
- Potential adverse impacts related to culture pathogenicity can occur.
- Microorganisms may not adapt to survive or there may be insufficient contaminant concentrations to support growth.
- Microorganisms may be consumed or outcompeted by other organisms already naturally present. The full microcosm must be understood.
- There is the possibility of deleterious metabolite(s) production.
- Bioaugmentation is not suitable for most inorganic contaminants.
- High contaminant concentrations may be toxic for microorganisms.
- Efficiency is affected by presence of metals.
- VC and/or methane concentrations may become vapor intrusion concerns.
- Biofouling may occur.

Other Considerations

- Identifying existing populations of indigenous microorganisms that are suitable for bioaugmentation at the site is pertinent.
- Nutrient requirements of microorganisms should be evaluated.
- Competitive or undesirable microbial activities should be minimized.
- Appropriate microbial culture should be identified based on target contaminants.
- Culture integrity should be maintained while producing sufficient quantity of organisms.
- Survival and performance of the added organisms should be monitored.
- Certain amendments may have patent restrictions.
- Explosive conditions may form due to methane production during reductive dechlorination.
- Microbial consortia can be used to target multiple COCs in certain cases.

Health and Safety

- Pressured line hazards can occur.
- Exposure to microorganisms may pose risks to human health.

Additional Information

- ([USEPA 2000](#)) [Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications](#).
- ([USEPA 2001](#)) [Use of Bioremediation at Superfund Sites EPA 542-R-01-019](#)
- ([Government of Canada 2019](#)) [Bioaugmentation Fact Sheet](#)

A2 Abiotic Amendments

This section describes abiotic amendments that are used in abiotic degradation of contaminants. In some cases, the conditions created to encourage biological breakdown of contaminants will also be conducive to abiotic chemical transformation of the contaminants, which occurs without the help of organisms (Cwiertny and Scherer 2010). Added oxygen will oxidize many compounds without biological catalysis, and hydrolysis of organic contaminants can happen spontaneously. Zero-valent iron can be added to support anaerobic bioremediation by producing hydrogen as it oxidizes and to abiotically reduce contaminants.

A2.1 Chemical Oxidants

ISCO is the delivery of an oxidant to the subsurface to degrade or transform COCs. ISCO can be used for treating the vadose and saturated zones. ISCO is effective at treating a wide variety of contaminants at both the source area and within the aqueous plume (although it is more cost-effective per mole of contaminant at higher contaminant concentrations), and often

produces decreased groundwater COC concentration results within weeks to months of the time of application.

Common oxidizing agents include catalyzed hydrogen peroxide (CHP, sometimes called modified Fenton's reagent), ozone, peroxone (the combination of ozone and hydrogen peroxide), activated and catalyzed persulfate, sodium percarbonate, and sodium or potassium permanganate. These oxidants are supplied in various forms. ISCO is amenable to treat groups of organic compounds (for example, BTEX, MTBE, CVOCs, SVOCs, carbon tetrachloride, select pesticides (for example, DDT, chlordane, lindane, etc.)), and some energetics (for example, dinitrotoluene or DNT, trinitrotoluene or TNT, and hexahydro-1,3,5-trinitro-1,3,5-triazine or RDX). ISCO is less effective on PAHs, PCBs, DCA, pesticides, chloroform, and metals that require reducing reactions and radicals. Some oxidants have narrow ranges of target compounds; for example, permanganate can attack only double bonds in compounds like PCE or TCE.

Limitations

- Certain amendments may cause exothermic reactions, leading to high temperature and pressure conditions.
- Amendments may cause high pH conditions problematic for biological growth.
- ISCO may not be economically feasible for low concentration large plumes.
- Metals impurities such as hexavalent chromium may be present in certain commercial-grade amendments and while in the reduced state can be reoxidized.
- Certain metals may be mobilized.
- Certain impurities or byproducts (for example, acetone, 2-butanone, etc.) may be introduced into groundwater as a result of the oxidation reactions.
- Byproducts from reactions can cause permeability reduction in the subsurface.
- Post-treatment contaminant rebound may occur.
- Certain amendments have a short life span, limiting migration in the subsurface.

Other Considerations

- Oxidant demand can depend on contaminant mass and distribution, presence of NAPL, geochemical conditions, SOD/NOD of treatment zone, and presence of oxidant scavengers.
- Specialized delivery systems may be required.
- Groundwater quality impacts from byproducts may trigger monitoring requirements.
- Certain amendments may have patent restrictions or regulatory restrictions due to ancillary components of the amendment, such as salts and metals.
- VOCs may volatilize during exothermic reactions.

Health and Safety

- Exothermic reactions could pose an explosive hazard.
- Health hazards may occur due to fugitive gas and dust emissions.
- Spills and releases onto combustible materials can cause fires.
- Pressurized line hazards may occur.
- Health and safety hazards related to handling of amendments should be considered.
- Oxidants and other amendments/activators should be stored with compatible chemicals, and material compatibility should be considered for the injection system components to avoid potential adverse reactions, failure of seals, etc.
- Consider applicable federal, state, and local regulations (for example, DOT, fire department) for transportation and on-site storage requirements.

Additional Information

- (ITRC 2005) ITRC In Situ Chemical Oxidation of Contaminated Soil and Groundwater
- (SERDP 2006a) In Situ Chemical Oxidation for Groundwater Remediation – Technology Practices Manual

A2.2 Chemical Reducing Compounds for Degradation Enhancement

In situ application of reducing compounds can degrade or chemically transform a variety of contaminants from toxic compounds to potentially nontoxic compounds. One example is provided in A Citizen's Guide to In Situ Chemical Reduction (USEPA 2012a). A second example is when ZVI comes in contact with TCE; the TCE is transformed to chloroacetylene and sometimes acetylene. Remediation using reducing compounds can decrease the concentrations of halogenated ethenes and ethanes, dinitrotoluene, and energetics, and transform some metals such as chromium (VI) and uranium (VI) and other

metalloids to less toxic and less mobile forms. The most commonly used reductant is ZVI, which is used to remediate halogenated ethenes and ethanes, energetics, and some metals/metalloids (chromium (VI), arsenic, and uranium) (ITRC 2011c). Other reductants that are used to address metals include ferrous iron, sodium dithionite, sulfide salts (calcium polysulfide), and hydrogen sulfide (Dresel et al. 2011). The introduction of substrates to microbially produce reducing conditions favorable to microbial reduction of iron and sulfates also has been used to treat dissolved metal contamination (Waybrant et. al. 2002). Because of their higher surface areas, nano-ZVI particles are more reactive than larger ZVI particles. However, nano-ZVI particles can be consumed more rapidly by reaction with oxygen, nitrate, and sulfate, and won't persist in the environment as long as larger ZVI particles and generally are more expensive.

Limitations

- In situ application of reducing compounds is applicable for treatment of dissolved phase and soil phase if adequately distributed.
- ZVI as a reducing agent imposes a risk of hydraulic short-circuiting and aerobic iron corrosion. High concentrations of nitrate, sulfate, carbonate, or acidic conditions can corrode the ZVI or form precipitates coating the ZVI, thereby shortening the expected life span of the ZVI.

Other Considerations

- Metals are typically treated under alkaline conditions.
- Degradation of VOCs can occur via biotic or abiotic pathways.
- Combined reductants with sulfides and carbon substrates may be used.
- A multimechanism environment for reduction, adsorption, precipitation, and sequestration of heavy metals is currently being considered.
- ZVI-based amendments and other zero-valent metals that can be used as reducing agents are currently being studied.
- Influence of ZVI in the subsurface can lead to hydrogen production, which can promote growth of anaerobic microorganisms and enhance natural reductive degradation.
- ZVI can be combined with biosubstrates and bioaugmentation.

Health and Safety

- Hazards related to handling of the reducing agents must be considered.
- Depending on emplacement technique, pressurized line hazards or general heavy equipment hazards can occur.

Additional Information

- USEPA In Situ Chemical Reduction Overview Web Page

A2.3 Biogeochemical Transformations

The term "biogeochemical transformation" (BGT)" collectively describes the physical, chemical, and biological processes induced by reduced iron minerals in the subsurface, which transform contaminants into nontoxic daughter compounds. For instance, the reactive iron(II) sulfide degrades tetrachloroethylene (PCE) into ethene via the abiotic chemical reaction described below (NAVFAC 2015).

$$C_2Cl_4 + FeS + 4H_2O = C_2H_2 + Fe^{+3} + SO_4^{-2} + 4Cl^- + 6H^+$$
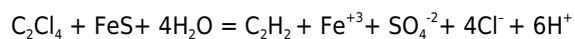
Other final degradation products including acetylene, ethene, and ethane can be formed from the reaction of PCE with ferrous sulfide. For this application, reduced iron minerals may be derived from naturally occurring geological formations or be formed by microbial activity under anaerobic conditions. Reduced iron minerals that have been exploited for remediation purposes include iron sulfides such as mackinawite (FeS), pyrite (FeS_2), magnetite (Fe_3O_4), and green rusts. Iron sulfides are often found naturally in anaerobic sediments such as wetlands and salt marsh environments. Green rusts are found naturally in soils and sediments in suboxic (low levels of oxygen and high levels of sulfur are present simultaneously) and anoxic (no oxygen) conditions. Iron sulfides, magnetite, and green rust have been studied the most because they are the most reactive. Among these three types of iron minerals, iron sulfides are the best understood.

For an in situ BGT application to be successful, there must be a balance between iron, sulfate, and electron donor in the system. Depending on the site conditions (determined through mineralogical studies, geochemical data, and/or microcosm studies), one or more of the three amendments may be required for the site. When using bioreactors and trench biowalls, solid amendments like mulch are typically emplaced. For injection approaches, liquid amendments are used. (For a more

detailed description of the common amendments used for biogeochemical transformations, as well as targeted contaminants, please refer to ([ESTCP 2005a](#)).

Limitations

- Sulfate reduction requires neutral pH and abiotic transformation requires higher pH levels; therefore, careful monitoring of pH. is required.
- Iron fouling may occur.
- Less reactive FeS_2^- may form instead of FeS.
- Reactions may not be complete or may be slow.

Other Considerations

- Required subsurface conditions include high sulfate concentrations, high DOC, and presence of sufficient iron oxide minerals. These may be naturally present or introduced.
- Biogeochemical transformation is most efficient in anaerobic conditions and under low levels of naturally occurring biodegradation.
- Sufficient residence time for the amendment should be considered during design to allow for both sulfate reduction and abiotic transformation of contaminants.

Health and Safety

- Pressurized line hazards may occur.

Additional Information

- ([NAVFAC 2015](#)) Biogeochemical Transformation Handbook
- ([Yongtian 2010](#)) Identification and Characterization Methods for Reactive Minerals Responsible for Natural Attenuation of Chlorinated Organic Compounds in Ground Water

A2.4 Activated Carbon–Based Injectates

Carbon-based injectates (CBI) are in situ remediation amendments for contaminated soil, sediment, and groundwater. To differentiate these products from other organic carbon–based amendments frequently used for in situ remediation of chlorinated solvents, EPA refers to the technology as activated carbon–based technology. The activated carbon-based injectates (ACBI) are based on an adsorptive capacity of activated carbon (AC). The AC contains microscopic pores (micropores) that increase the surface area available for adsorption and chemical reactions. The AC adsorbs organic chemicals in the micropores through Van der Waals forces. Adsorption itself does not eliminate contaminants, but rather it limits migration. The primary mechanism for this media is typically sorption. Degradation of the compounds occurs by a secondary process (biotic or abiotic) on the media. Desorption is driven by the concentration gradients between the ACBI and the aquifer matrix. For contaminants not amenable to biotic or abiotic degradation, the sole treatment mechanism is adsorption of the contaminant.

The injectate is a mixture of powdered or pulverized AC, water, and additives such as electron acceptors, nutrients and microbes, or oxidants ([Performance of Injected Powdered and Liquid Activated Carbon at a Petroleum Hydrocarbon Site](#)). The AC adsorbs and concentrates the contaminants, which are then treated by additives within the mixture. Common ACBI amendments include gypsum to support sulfate-reduction breakdown of hydrocarbons or a soluble substrate to support anaerobic dechlorination or iron for abiotic breakdown of solvents.

A variety of secondary degradation mechanisms such as aerobic/anaerobic bioremediation and chemical reduction/oxidation can occur on the sorbed contaminants. The CBI amendments also provide a favorable environment for the natural processes, such as microbial degradations of contaminants.

Limitations

- Treatment effectiveness is limited by adsorptive capacity of the amendment, especially for contaminants not amenable to biotic or abiotic degradation.
- Contaminant flux into site may overwhelm rate of adsorption/degradation.
- Competitive adsorption may displace weakly sorbed compounds over time.

Other Considerations

- Long-term effectiveness data are currently lacking.

- Poor distribution of slurry-type amendments can occur in high or low permeability materials.
- Nutrients or amendments added to foster a biodegradation process will become depleted (some rather quickly), and there must be plans to monitor and periodically add more "food."

Health and Safety

- Pressurized line hazards may occur.
- There is a potential for generation of fine particulates (carbon dust) and inhalation hazard during on-site slurry preparation or alternative emplacement techniques (for example, placement of dry carbon in excavation or trench).

Additional Information

- (USEPA 2018c) Remedial Technology Fact Sheet – Activated Carbon-Based Technology for In Situ Remediation

A2.5 Surfactants and Co-Solvents via Solvent Flushing

In some cases, surfactants are used to enhance solubility and bioavailability of contaminants from soil and sediments to improve treatment efficiency (West 1992; Rouse 1996; Perolo 2010). Solvent flushing involves injection of an alcohol/water solution (10–50 vol. %) to increase the NAPL solubility within the aqueous phase. Some alcohols may also result in a reduction of the NAPL/water interfacial tension and mobilize the NAPL. The aqueous solvent solution is injected into the subsurface so that it flows through the contaminated area. The solvent and dissolved contaminants are subsequently extracted and can be treated aboveground or sent off-site. As shown in Table 3-2, common surfactants used for in situ remediation applications include anionic surfactants, cationic surfactants, and electrolytes and co-solvents.

The primary mechanism for recovering NAPLs by using surfactants is either mobilization or solubilization. Surfactants can form micelles that can contain minute droplets of NAPL; hence the term "solubilization" is used to describe the apparent increase in NAPL solubility within the aqueous phase instead of the term "dissolution." Although the two terms and mechanisms are different, the approach for using the solubilization mechanism is similar to that of dissolution, or pump and treat, where the recovery of NAPL is achieved by continuously injecting the surfactant formulation and producing solubilized NAPL. The degree of solubilization is typically two orders of magnitude higher than aqueous solubility, so a concomitant decrease in remediation time is achieved compared to standard groundwater extraction techniques. This approach has been called surfactant-enhanced aquifer remediation (SEAR) and differs from the surfactant flooding approach that relies upon mobilization as described below.

Surfactant formulations can also be used to reduce interfacial tension, which is the primary mechanism trapping NAPLs within the porous media. Such a formulation will allow the NAPL to become mobile and to be recovered from the subsurface. The surfactant used to solubilize vs. mobilize is often the same, as are the co-surfactants and other ingredients in the formulation. The behavior of the formulation, and hence whether it is used to solubilize or mobilize, is affected by parameters such as temperature, salinity, and other factors.

More background on surfactant phase behavior can be found in (Schechter. 1988; NFCSC 2002). Surfactants and solvents are useful to either increase apparent solubility or mobilize NAPLs. They are not used for dissolved groundwater plumes. NAPLs that have been candidates for surfactant or solvent action include TCE and PCE, creosote, gasoline, jet fuels, coal tar, and PCBs. In some cases; only laboratory scale feasibility studies were conducted, while in other cases a full-scale field implementation was completed.

Limitations

- There is a limited record of success for the in situ application of this technology.
- Surfactants may not recover dissolved components.
- Surfactants may not be compatible with most inorganic contaminants.
- Surfactants may not be suitable for soils rich in cationic materials or high organic content.
- This method may be less effective in low permeability and heterogeneous soils.
- Use of surfactant could increase dissolved phase contamination and NAPL plume size.
- This method requires pumping of groundwater to capture or hydraulically control NAPL.

Other Considerations

- Selection of surfactants should be made on a site-by-site basis because a wide range of surfactants is available.
- On-site or off-site treatment of recovered NAPL is required.
- Design should consider conditions to ensure capture of mobile NAPL.

- The high concentration of recovered NAPL can overwhelm existing on-site treatment systems.
- Cationic surfactants have not been used due to high surface adsorption.
- Some co-solvents, such as isopropanol, can result in byproducts. Separation of NAPL and surfactant to allow reuse of surfactant may make the process more economical. A treatment process to remove surfactant and co-solvent must be considered.
- Depending on the makeup of the amendment, there could be regulatory restrictions that must be met.

Health and Safety

- Solvents, surfactants, and co-surfactants are flammable.
- Relevant Safety Data Sheets should be consulted for proper PPE, handling, and storage requirements.

Additional Information

- (ITRC 2003) Technical and Regulatory Guidance for Surfactant / Cosolvent Flushing of DNAPL Source Zones
- (NFCSC 2002) Surfactant-Enhanced Aquifer Remediation (SEAR) Design Manual

A3 Other additives

To stimulate microbial growth and degradation of contaminants, supplemental amendments including those that directly support microbiological growth (C, N, P) and those that maintain or create favorable geochemistry (pH buffering, dissolved O_2) are used. Injection of small amounts of commercially available methane inhibitors is also recommended to retard the proliferation of methanogens to ensure the complete mitigation of any potential methane issue, as summarized below in A3.3.

A3.1 pH Buffers

At a field site, pH is influenced by a complex relationship between organisms, contaminant chemistry, and physical and chemical properties of the local subsurface environment. For example, in low-alkalinity systems, fermentation of complex substrates generates acids, and hydrochloric acid (HCl) is formed during anaerobic dechlorination. These processes may significantly decrease groundwater pH. Reducing groundwater pH to below 5 will likely inhibit microbial growth (for example, sulfate reducers, methanogens, and most dechlorinating microbes) (Maillacheruvu 1996). Normally, the natural buffering capacity of the aquifer matrix, as measured by alkalinity, is adequate to prevent the development of acidic groundwater pH; however, at some sites, pH buffer amendments may be required to maintain near-neutral pH in groundwater systems with insufficient natural buffering capacity. The maintenance of near-neutral groundwater pH is not only important for microbial growth, but also for secondary groundwater geochemistry.

For many acidic aquifers, pH buffering will be required to bring the pH into a range of 6–8, which is favorable for reductive dechlorination. Various pH adjustment agents have been used, including soluble materials such as sodium or potassium bicarbonate, sodium carbonate, sodium hydroxide, calcium hydroxide (slaked lime), or less soluble materials such as calcium carbonate, calcium oxide, magnesium oxide, magnesium hydroxide, dolomitic hydrated lime, and limestone. Bicarbonates have the lowest pH for saturated solutions of these buffers, but also have the least buffering capacity and the potential for carbon dioxide production. Calcium carbonate or limestone is practically insoluble, but has an equilibrium pH of 9.4. The other soluble reagents, including sodium hydroxide, sodium carbonate, and calcium hydroxide, have higher pH equilibrium levels and could therefore overshoot the desired pH range. The less soluble buffers are more difficult to deliver and generally have high equilibrium pHs (TerraSystems unpublised Report).

Common buffers include $CaCO_3$ (calcium carbonate), MgO (magnesium oxide), $Mg(OH)_2$ (magnesium hydroxide), $KHCO_3$ (potassium bicarbonate), $NaHCO_3$ (sodium bicarbonate), CaSx (calcium polysulfide), $FeSO_4$ (ferrous sulfate), $FeCl_3$ (ferric chloride), MgO (magnesium oxide), and CaO (calcium oxide).

A3.2 Nutrients

An aquifer normally contains sufficient amounts of nutrients for microbial growth. In engineered bioremediation; however, due to the presence of the contaminants or the addition of organic substrate, the nutritional demand imposed by rapid microbial growth may exceed the capacity of the aquifer system (Chamberlain 2003). In addition to a readily degradable carbon source, microorganisms also require nutrients such as N, P, and K for cellular metabolism and growth (Bamforth 2005). Commonly used nutrients include mineral salts (for example, KNO_3, $NaNO_3$, $Ca(NO_3)_2$, NH_4NO_3, $(NH_4)_2SO_4$, K_2HPO_4, $(NH_4)_2HPO_4$, $MgNH_4PO_4$,), anhydrous ammonia (NH_3), urea ($(NH_2)_2CO$), and many commercial inorganic fertilizers. In practice,

nitrogen and phosphorus requirements are often estimated by calculating a carbon to nitrogen to phosphorus ratio C:N:P close to 100:(10–5):1 (Atlas 1981; Atlas 1992). In practice, although most aquifers contain the necessary nutrients for microbial growth, nutrients are often added as an extra measure of assurance, and because they are generally inexpensive. Dechlorinating bacteria such as *Dehalococcoides* do not produce vitamin B_{12}. Dechlorination efficiency can be increased by adding vitamin B_{12} to the aquifer. (He 2007; Harkness 2012) demonstrated the statistical value of nutrient addition in microcosm studies evaluating DNAPL TCE biodegradation.

A3.3 Methane Inhibitors

Methane concentrations may increase due to the proliferation of methane-producing bacteria, which occurs when high levels of carbon substrate such as emulsified vegetable oil are introduced into the subsurface. Methane can be metabolized by many microbes to carbon dioxide under aerobic conditions in the vadose zone. To ensure the complete mitigation of any potential methane issue, injection of small amounts of commercially available methane inhibitors is recommended to retard the proliferation of methanogens. "The methane inhibitor Provect-CH4 is a food-grade, natural source of Monacolin K (otherwise known as Lovastatin) that is used to prevent methane production by inhibiting the growth and proliferation of methanogenic Archaea. In environmental remediation applications, it can be used as a supplement to EISB and ISCR amendments, rendering them safer and more effective." (Provectus 2014) It is supplied as a water-soluble powder that can be mixed on site and added in conjunction with the electron donor (NAVFAC 2018).

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