Enhanced In Situ Reductive Dechlorination for Groundwater

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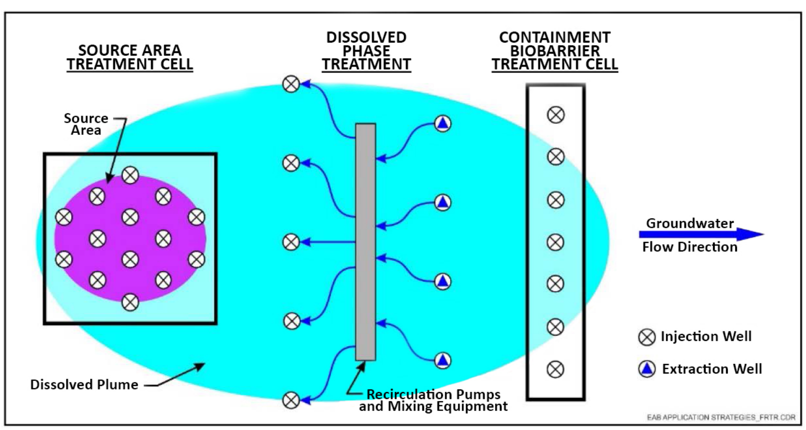
Schematic



Enhanced In Situ Bioremediation

Introduction

Enhanced in situ reductive dechlorination (ERD) is the process of modifying chemical, physical, and biological conditions in the aquifer to stimulate the microbial degradation of contaminants under anaerobic conditions to harmless end products (e.g., carbon dioxide [CO_2] or ethene). It is used to remediate volatile organic compounds (CVOCs) (e.g., trichloroethylene [TCE]) and certain pesticides and other chlorinated organic contaminants. ERD commonly is used to treat dissolved phase plumes downgradient of source areas, but also can be designed to treat CVOC source areas including those that contain dense non-aqueous phase liquid (DNAPL).

Other Technology Names

Biostimulation
Bioaugmentation
Enhanced anaerobic bioremediation
Enhanced Natural Attenuation
Halorespiration
Dehalorespiration

Description

ERD is the process through which chlorine atoms attached to an organic compound are sequentially removed under anoxic (no oxygen) conditions. Application of ERD is comprised of biostimulation and sometimes bioaugmentation to modify existing geochemical and biological conditions in an aquifer to facilitate degradation of contaminants (AFCEC, NAVFAC, ESTCP, 2004). Biostimulation refers to the introduction of an electron donor (carbon source) into the aquifer for stimulating microbial growth. The carbon source is used as food by native microbes, which in turn, produce hydrogen through fermentation reactions. This process depletes the aquifer of dissolved oxygen (DO) and other electron accepters including nitrate, sulfate, and ferric iron, which lowers the oxidation-reduction potential (ORP), thereby creating the conditions for reductive dechlorination to occur (EPA, CLU-IN, 2016; NAVFAC, 2007a).

Bioaugmentation refers to the introduction of microbes, which may be required at some sites to accelerate the acclimation and biodegradation process if the existing microbial population is not adequate at the onset of treatment (ITRC, 2002; ITRC, 2008b). At many sites, microbes capable of dechlorinating cis-1,2-

dichloroethene (cis-1,2-DCE) and vinyl chloride (VC) are not present in sufficient quantities resulting in a condition referred to as "cis-1,2-DCE and VC stall" (NAVFAC, 2007b). In those cases, bioaugmentation can be used in combination with biostimulation to achieve the necessary conditions to overcome stall and promote complete reductive dechlorination (NAVFAC, 2013). Under the right conditions, ERD has been proven successful as a remedial strategy to treat chlorinated solvent source areas, including those that contain low to moderate levels of DNAPL mass (ITRC, 2007; ITRC, 2008a). However, ERD is more commonly used to treat dissolved phase plumes downgradient of the source area, or to create reactive barriers to prevent further migration of a plume.

Reaction Mechanisms

ERD can occur through several reaction (degradation) mechanisms including:

- **Direct Anaerobic Reductive Dechlorination** is a biological process where microbes receive energy and grow through sequential reductive dechlorination. In this reaction, chlorinated compounds serve as the terminal electron acceptor, and hydrogen (sometimes acetate as well) serves as the electron donor. Hydrogen is produced by fermenting populations that utilize the added organic substrate. Anaerobic reductive dechlorination can also be referred to as halorespiration or dehalorespiration. A common anaerobic dechlorination pathway is the degradation of tetrachloroethene (PCE) to TCE to *cis*-1,2-DCE and small amounts of *trans*-DCE to VC to ethene (Lorenz and Loffler, 2016). The chlorinated ethene molecule is the electron acceptor in this reaction. Although fermentation products such as acetate may serve as an electron donor, hydrogen is recognized as the predominant electron donor for anaerobic dechlorination of CVOCs. Following similar degradation pathways, chloroethanes and chloromethanes may also be sequentially reduced by anaerobic dechlorination. For chloroethanes, 1,1,1- trichloroethane (TCA) is reduced to 1,1-dichloroethane (DCA) then chloroethane (CA) and finally to ethane. For chloromethanes, carbon tetrachloride (CT) can be reduced to chloroform (CF) then methylene chloride (MC) to chloromethane (CM) and finally to methane (Stroo, Lesson et al., 2013; ITRC, 2002).
- **Cometabolic Anaerobic Reductive Dechlorination** is a process where chlorinated compounds are reduced by non-specific enzymes or co-factors that are generated for metabolism by another compound (i.e., the primary substrate) in an anaerobic environment. Cometabolism does not produce energy for or benefit the microbe producing the non-specific enzyme or co-factor. To sustain the cometabolic process, sufficient primary substrate is required to support the microbe producing the non-specific enzyme or co-

factor (Stroo and Ward, 2010; ITRC, 2008b). Reliance on this indirect strategy can lead to the accumulation of toxic end products such as cis-dichloroethene (cDCE) and vinyl chloride (VC) as cometabolism has slower reaction rates than direct reductive dechlorination (ITRC, 2002).

- **Abiotic Reductive Dechlorination** is a chemical degradation reaction where a chlorinated hydrocarbon is degraded (i.e., reduced) by reactive compounds, such as metal sulfides. By definition, the reaction is not microbially mediated. Adding organic substrate to an aquifer lowers the redox environment and can produce ferrous iron and hydrogen sulfide through biologically-mediated iron and sulfate reduction, respectively. It is the reactive compounds that abiotically reduce the chlorinated solvents (Stroo, Lesson et al., 2013; Stroo and Ward, 2010). More information is provided in the In Situ Biogeochemical Transformation Processes profile.

Distinguishing between the various pathways at sites is difficult and often not possible given all the variables in the subsurface. As such, all three pathways are recognized to concurrently contribute to enhanced anaerobic degradation.

Common Amendments

All of the above processes generally require the introduction of an electron donor to produce the necessary conditions for biodegradation to occur (AFCEC, NAVFAC et al., 2010; Borden, 2006). These electron donors/carbon sources can be separated into three principal groups based on their physical characteristics including:

- **Aqueous (soluble):** Aqueous compounds are highly soluble and can be more readily distributed across large areas by groundwater flow/advection, assuming reasonable flow velocity. However, they also are readily bioavailable, and, therefore, are consumed in a relatively short time. Examples of soluble substrates include lactate and fatty acids, butyrate, methanol, ethanol, benzoate, molasses, and high fructose corn syrup.
- **Slow-Release:** Slow release compounds tend to have low solubility limits and greater viscosities than their aqueous counterparts, making them more difficult to emplace in the aquifer. However, because they are less soluble (and less bioavailable), they persist much longer. This slow-release aspect allows these substrates to serve as long-lasting sources of electron donor and minimize the number of substrate injections performed at a site. Their properties enables the substrate to remain relatively immobile in the subsurface. Viscous fluid substrates, including emulsified and neat vegetable oils and hydrogen release compounds, are included in this category (ESTCP, 2009).

- **Solid Substrates:** Typical solid phase substrates, consisting of materials such as mulch, compost and chitin, are used either as a one-time amendment or in biobarrier construction. Degradation of the substrate by microbial processes provides a number of breakdown products, including metabolic and humic acids, which act as secondary fermentable substrates.

At many sites, the aquifer is bioaugmented with commercially available microbial consortia consisting of one or more of *Dehalococcoides* (DHC), *Dehalobacter*, sulfate reducers, methanogens, and fermentative microbes, which can degrade chlorinated ethene, chlorinated ethane, and mixed plumes. It has been found that the microbial population must also contain necessary functional genes to facilitate the ERD process. For example, DHC must contain the function gene vinyl chloride reductase (*vcrA*) to degrade VC or a "stall" condition will occur (Stroo, Lesson et al., 2013; Lorenz and Loffler, 2016).

Microbial cultures should be added only after the necessary redox conditions have been achieved in the aquifer. DCE and VC require stronger anaerobic conditions (i.e., low redox conditions) than TCE for the ERD process to proceed to completion (e.g. to ethene) or a "stall" condition may occur (NAVFAC, 2007b). The cost to apply the microbes may be in the same range as additional characterization required to evaluate if the necessary microbial community is present on site in sufficient concentrations (Stroo, Lesson et al., 2013). As a result, many times bioaugmentation is designed as part of the ERD remedy and performed after biostimulation to attain the necessary redox conditions to ensure survival and proliferation of the microbial community.

Various microbial tools are available that can be used in combination with other analytical methods to establish baseline conditions to determine biostimulation design requirements, evaluate the potential benefits of bioaugmentation, and monitor remedy progress by comparing changes in baseline versus post-treatment conditions. The link to the NAVFAC Web tool in the Resource Section provides additional information (NAVFAC, 2007c; Lorens and Loffler, 2016).

In some cases, it may be necessary to add additional nutrients for microbial growth, such as vitamins (e.g., research to date has found vitamin B12 to be key facilitator of ERD process), nitrogen, phosphorous, and yeast extracts. In addition, it also is generally necessary to add a buffering agent, such as bicarbonate, to mitigate a decrease in aquifer pH, which occurs as a result of fermentation and liberation of hydrogen ions. Typically, buffers are water soluble and distribute easily through the aquifer. However, they can become easily diluted and consumed, potentially necessitating re-application (EPA, 2000; ITRC, 2008b).

Amendment Delivery Approaches and Design Considerations

There is a wide range of amendment application techniques depending on the physical properties of the amendments and the characteristics of the area to be treated (i.e. source area or dissolved phase plume) and remedial objectives (e.g. prevent further plume migration). Delivery techniques include direct push methods through wells or points and recirculation through horizontal or vertical extraction and injection wells. Combined approaches consisting of direct push in some locations and recirculation in others are sometimes used (NAVFAC, 2015). Detailed information and best practices to introduce and distribute amendments into the aquifer can be found here.

Understanding the characteristics of the subsurface is important when applying ERD. Distribution tends to be easier in sandy, more permeable and homogeneous aquifers. It is difficult to obtain adequate distribution of amendments in fine sand, silt and clayey materials and in heterogeneous aquifers containing multiple discrete lithologic units. In less permeable aquifers, injection points must be placed closer together (NAVFAC, 2013). Geochemical conditions including hardness, total organic carbon, dissolved organic carbon, pH, buffering capacity, and contaminant concentration must be known to design the amendment distribution system and determining appropriate mass, concentration, and flowrate of amendments. The concentration of amendments used depends on these factors as well as the type of delivery approach applied. Many amendment vendor have developed dosing design tools to optimize an appropriate dosage based on site specific conditions for the amendments they sell (ITRC, 2008b).

Design considerations for ERD systems are site-specific and based on remedial objectives, regulatory requirements, and site-specific factors including size of the site, aquifer physical, chemical, and hydrogeologic properties, presence of DNAPL (NAVFAC, 2012; ITRC, 2002), and concentrations of contaminants of concern (COCs) among others. Economic limitations often are predominant design considerations for the treatment system size. In designing the injection strategy, a common goal is to reduce overall project cost by minimizing the number of injection wells and events, as well as minimize amendment costs. An optimized design can be obtained by balancing the physical site characteristics with the remedial objectives given a particular substrate. For example, the use of large volumes of soluble substrate may minimize the number of injection points, but the substrate itself may have a higher purchase cost and have higher operation and maintenance (O&M) costs due to excessive lowering of pH and/or biofouling in the wells or formation (ESTCP, 2005b).

Monitoring and Data Interpretation

A variety of process monitoring must be performed while introducing and distributing the bioremediation amendments. Flowrates and pressures are measured at the injection manifold and/or each injection well to control injection flowrates. Similar data is collected from extraction wells if a recirculation system is employed. Groundwater quality parameters such as pH, conductivity, oxidation-reduction potential (ORP); and groundwater elevations also are monitored routinely (NAVFAC, 2013). Results are used to evaluate distribution of the amendments as well as to determine hydrogeologic and geochemical changes that may occur in the aquifer as a result of the application. Although these changes typically occur within the treatment area and return to near baseline values shortly after the application, the aquifer geochemistry must be monitored to ensure that any nearby downgradient receptors are not impacted. Monitoring equipment usually consists of a groundwater quality meter equipped with a flow-through cell and a water level meter (Stroo, Lesson et al., 2013).

After application is complete, longer-term performance monitoring must be performed. This monitoring typically consists of measuring concentrations of COCs in monitoring wells at various time intervals to determine the degree of treatment and the need for additional injections. Groundwater quality also is measured and used as a line of evidence to evaluate aquifer conditions. In particular, ORP, DO, and pH are important indicators that the aquifer remains suitable for continued ORD or if additional amendments are required. Parameters that commonly are monitored are summarized in the table below. Other testing such as microbial assays and carbon isotope analysis can also provide additional lines of evidence of ERD versus dilution and can be used to project attenuation rates and remediation timeframes.

Measurement	Primary Purpose
Groundwater Elevations	Determine groundwater flow direction. If barriers are employed ensure they are not impacting groundwater flow direction
COCs	Monitor changes in plume size/location
Dissolved hydrocarbon gases (methane,	Evaluate remedial performance. An increase

ethane, and ethene)	in ethene is a good indicator of complete dechlorination occurring. Elevated levels of methane are indicative of highly reducing conditions and possible over-dosing of the carbon source.
Groundwater quality (DO, ORP, pH, conductivity and temperature) nitrate, sulfate, ferrous iron)	Determine conditions are present for DHC survival and proliferation
Anions (nitrate, sulfate, sulfide, ferrous iron, chloride)	Evaluate levels of competing electron acceptors and assess the redox state of the aquifer. Increases in chloride concentration is indicative of reductive dechlorination occurring.
Total organic carbon & volatile fatty acids	Determine distribution of EVO
qPCR for DHC and appropriate genes (e.g. *vcrA*)	Determine the viable population of DHC in groundwater and presence of necessary genes to completely degrade COCs to end products
Phospholipid fatty acid analysis	Provides understanding of microbial community
Dissolved Metals	Evaluate impacts to groundwater quality

Development Status and Availability

The following checklist provides a summary of the development and implementation status of ISCO:

☐ At the laboratory/bench scale and shows promise

☐ In pilot studies

☒ At full scale

☒ To remediate an entire site (source and plume)

☐ To remediate a source only

☒ As part of a technology train

☐ As the final remedy at multiple sites

☒ To successfully attain cleanup goals in multiple sites

ERD is available through the following vendors:

☒ Commercially available nationwide

☐ Commercially available through limited vendors because of licensing or specialized equipment

☐ Research organizations and academia

Applicability

Contaminant Class Applicability Rating for Enhanced Reductive Dechlorination

(Rating codes: ● Demonstrated Effectiveness, ◐ Limited Effectiveness, ○ No Demonstrated Effectiveness, I/D Insufficient Data, N/A Not Applicable)

Nonhalogenated VOC	Halogenated VOC	Nonhalogenated SVOC	Halogenated SVOC	Fuels	Inorganics	Radionuclides	Munitions	Emerging Contaminants
○	●	○	●	○	○	○	○	I/D

ERD is applicable at sites contaminated with PCE, TCE, TCA, CT, and other CVOCs (Stroo, Lesson et al., 2013; Stroo and Ward, 2010). These compounds are susceptible to reduction under anaerobic conditions by either biotic (biological) or abiotic (chemical) processes and are generally not susceptible to aerobic oxidation processes (with the possible exception of cometabolic degradation). Other chlorinated contaminants degradable under anaerobic conditions include chlorobenzenes, chlorinated pesticides (e.g., chlordane), and oxidizers (perchlorate and chlorate). PCBs and chlorinated cyclic hydrocarbons (e.g. pentachlorophenol) can be more recalcitrant to ERD. Bench- and pilot-testing should be performed to evaluate the efficacy of ERD to treat these compounds.

ERD is applicable at sites at which conditions are reducing and are near anoxic or such conditions can be achieved through the introduction of amendments. Sites that are aerobic or have little natural buffering capacity may not be amenable to ERD (NAVFAC, 2013).

ERD systems may be applied to treat source zone areas and dissolved-phase plumes considering the following:

- **Source Zone Treatment:** The objective is to either reduce mass discharge from the source zone or accelerate source mass removal. In this context, it is necessary that good distribution of injected amendments is feasible. DNAPL source areas can be treated; however, treatment is rate limited by the time it takes for DNAPL to dissolve into the aqueous phase and may not be cost effective given the total mass that must be degraded and ability to adequately distribute the needed volume of carbon mass and other amendments to contact the DNAPL mass (NAVFAC, 2004; ITRC, 2007; ITRC, 2008a).
- **Dissolved Phase Plume.** The dissolved phase plume may be treated by employing a plume-wide restoration approach or alternatively using biobarriers. The objective of a plume-wide design is to treat the entire dissolved phase plume over a shorter remediation timeframe. Given the size of some plumes, creating an anaerobic reaction zone across a broad area can result in significant cost. However, this approach may reduce the overall timeframe for remediation. Plume wide delivery systems typically are designed as a large injection grid. Recirculation between wells is also used to facilitate amendment distribution (AFCEC, NAVFAC et al., 2004; NAVFAC, 2013, Stroo and Ward, 2010).

Cost

ERD can be a very cost-effective technology if properly designed and applied. Similar to many in situ remediation technologies, the most critical cost factors are associated with the contaminant mass to be treated, the nature and extent of contamination (i.e. size of the treatment area), ability to adequately distribute that carbon source and other amendments to contact the contaminant mass to be treated, and number of injection points/wells required. As with all in situ technologies, application costs vary according to site conditions and contaminants. Major cost drivers include:

Upfront Costs

- Need for pilot studies or bench-scale tests to demonstrate effectiveness at a particular site
- Size of treatment area and treatment approach (e.g. biobarrier, direct injection grid, recirculation)
- Types and quantities of amendments required, which is dictated by the contaminant mass to be treated
- Number of injection points/wells required, which is dictated by the contaminant mass to be treated and site hydrogeologic conditions
- Equipment (including need for permeability enhancement techniques) and labor to introduce and distribute the amendments, which is dictated by the subsurface geologic heterogeneity and corresponding contaminant mass distribution, application method and duration of each injection event

Operation and Maintenance Costs

- Frequency of reapplication of amendments,
- Monitoring requirements after amendment addition,
- Treatment timeframe.

The list above highlights those costs dependencies specific to ERD and does not consider the dependencies that are general to most in situ remediation technologies. Click here for a general discussion on costing which includes definitions and repetitive costs for remediation technologies. A project-specific cost estimate can be obtained developed using an integrated cost-estimating application such as RACER® or consulting with a subject matter expert.

Duration

Full-scale implementation can typically run between 2 to 10 years depending on the approach taken and nature and extent of contamination. Treatment timeframes, however, can be decades for more complex remediation sites involving significant source area contaminant mass, large plumes, and/or high initial dissolved phase contaminant concentrations. At many sites, it is necessary to perform multiple applications of amendments to sustain the redox conditions and corresponding microbial populations as the effects of the amendments diminish. Since it is often difficult to attain drinking water remedial goals in groundwater through ERD treatment, alone, the duration will also be dependent on the interim objectives established for the termination of injections and transition to an alternative remedial approach such as MNA.

Primary factors that influence the duration of ERD include:

- RGs and remedial action objectives
- Treatment methodology (i.e., source area treatment, dissolved plume treatment, containment)
- Presence of LNAPL or DNAPL and initial concentrations of COCs
- Number of injection events required
- Ability to achieve uniform distribution and sustained concentrations of amendments in the aquifer

Implementability Considerations

The following are key consideration associated with applying ERD:

- Moderate to high levels of DNAPL and residual CVOC mass may be more cost effectively treated by other in-situ technologies (e.g., in-situ thermal treatment). Biodegradation occurs in the dissolved phase, whereas the dissolution of DNAPL into the dissolved phase can be a slow mass transfer process, especially within the capillary/smear zone where DNAPL and residual CVOC mass often accumulates. DNAPL and residual CVOC mass also can become immobilized in less permeable soil units over time through capillary forces and become even less accessible to mass transfer into the dissolved phase. This leads to a slow treatment process for higher levels of DNAPL and residual CVOC mass, resulting in continuing rebound to initial dissolved phase groundwater concentrations until enough of the mass has been degraded (NAVFAC, 2012; ITRC, 2007; ITRC, 2008a).
- Biofouling can clog the well screen, adjacent sand pack, and/or adjacent formation at the injection or recirculation wells. To prevent or mitigate biofouling, typical approaches include pulsed injection, well flushing with clean water to remove substrate, and the application of non-oxidizing biocides to control growth in the immediate vicinity of the well reduces clogging of nutrient and water injection wells.

- Preferential flow paths due to heterogeneous lithology or subsurface structures and utility corridors can severely influence amendment distribution and result in pockets of untreated CVOC mass.
- Amendment distribution becomes more difficult and may be infeasible for lower permeability clay, highly layered, or heterogeneous subsurface environments. Higher injection pressures or permeability enhancement techniques (e.g., hydraulic or pneumatic fracturing) may be necessary to improve distribution of amendments for these conditions.
- Sites having high concentrations of heavy metals, highly chlorinated organics, long chain hydrocarbons, DNAPLs or inorganic salts can be toxic to microbes and therefore inhibit ERD (ITRC, 2007, ITRC, 2008a)
- Low temperatures in shallow groundwater in cold climates are known to slow bioremediation.
- Depending on site conditions, some CVOC daughter products may not be completely transformed to innocuous products. Microbial populations capable of reductive dechlorination of PCE and TCE to cis-DCE are relatively ubiquitous in the subsurface. However, the microbial populations may not be adequate to support the complete sequential degradation from *cis*-1,2-DCE through to ethene. When cis-1,2- "DCE/ VC stall" is observed at a site, several factors have been identified as possible contributors (NAVFAC, 2007b; Lorenz and Loffler, 2016):
    - Lack of the required DHC populations or corresponding functional genes required to complete the degradation of *cis*-1,2-DCE or VC.
    - Insufficient volume of electron donor/carbon source delivered that leads to mildly anaerobic conditions that will support the conversion of PCE/TCE to *cis*-DCE, but not the conversion of cis-DCE or VC to end products by ERD.
    - A shift in the ratio of parent CVOCs (e.g., PCE and TCE) to daughter products (e.g., *cis*-1,2-DCE and VC), because parent compounds can degrade at a faster rate than their daughter products. This leads to an increase in concentrations of daughter products and gives the appearance of a stall condition.
- ERD usually requires an acclimated population of microbes, which can take a few months or more; this presents a challenge in the case of recent spills or for recalcitrant compounds.
- ERD may not be effective at sites having oxic aquifers and/or relatively fast groundwater flow because a prohibitive mass of amendments might be required to achieve the necessary conditions to promote degradation.
- Application of ERD may depress the pH of the aquifer, increasing the mobility of various metals including arsenic.

Resources

AFCEC, NAVFAC, ESTCP. Addendum to Principles and Practices Manual – Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation. ESTCP Project ER-200627 (2010)
Enhanced in situ anaerobic bioremediation involves the delivery of organic substrates into the subsurface to stimulate anaerobic degradation of contaminants in groundwater. Effective application of the technology depends primarily on the delivery of appropriate levels of organic substrate in the subsurface and the development of optimal geochemical and oxidation-reduction (redox) conditions for anaerobic degradation processes to occur. Determining an appropriate substrate loading rate and an effective distribution method for the various substrate types commonly applied is a critical design and operational objective.

AFCEC, NAVFAC, ESTCP. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. ESTCP Report: Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. ESTCP Project ER-200627. (2004) (PDF) (457 pp, 6.5 MB)
This document is intended to assist the Air Force Center for Engineering and the Environment (AFCEE), Naval Facilities Engineering Service Center (NFESC), Environmental Security and Technology Certification Program (ESTCP), U.S. Army Corps of Engineers (USACE) and their U.S. Department of Defense (DoD) technology-transition partners in evaluating and applying enhanced in situ anaerobic bioremediation for restoration of groundwater contaminated with chlorinated solvents.

Borden, R. Protocol for Enhanced In Situ Bioremediation Using Emulsified Edible Oil, ESTCP Project ER-200221. (2006)
The objective of this protocol is to provide guidance on the use of emulsified edible oils for enhanced in situ anaerobic bioremediation.

EPA. CLU-IN Bioremediation of Chlorinated Solvents Web Site. (2016)
This web page is part of the CLU-IN Technology Focus area which consolidates information for specific technologies (in this case bioremediation of chlorinated solvents) in the following categories: Overview, Guidance, Application, Training, and Additional Resources.

EPA. Introduction to In Situ Bioremediation of Groundwater (542-R-13-018). (2013)
Provides an overview of in situ bioremediation and serves as a reference for designers and practitioners.

EPA. Guidance Memo: Applicability of RCRA Section 3020 to In Situ Treatment of Groundwater. (2000)
Clarifies that re-injection of treated groundwater to promote in situ treatment is allowed under Resource Conservation and Recovery Act (RCRA) Section 3020(b) if certain conditions are met.

ESTCP Cost and Performance Report: Loading Rate and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation (2010).
Provides approaches and case studies for determining substrate loading rates and the impacts of substrate delivery for ERD.

ESTCP Tool: Emulsion Design Tool Kit (2009)
The Emulsion Design Tool is a spreadsheet-based tool developed to guide the design of emulsified oil distribution systems for enhancing bioremediation of contaminants in groundwater. Also available is an overview presentation on implementing the tool as well as a User's Guide detailing necessary calculations and the effects of different design parameters on contact efficiency.

ESTCP Report: Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs. (2005a).
The objectives of this white paper are to 1) summarize the current status of this rapidly evolving innovative technology, 2) identify the key issues confronting the science, and 3) evaluate the lessons learned from current practical applications. In particular, this review is intended to be useful to remedial project managers faced with selecting, designing, and implementing a bioaugmentation strategy.

ESTCP Report. A Review of Biofouling Controls for Enhanced In Situ Bioremediation of Groundwater. (2005b)
The objective of this report is to review well rehabilitation and biofouling controls that are potentially relevant to ERD applications and to identify promising biofouling controls for comparative field evaluation and validation under ESTCP Project ER-0429.

ITRC. A Systematic Approach to In Situ Bioremediation in Groundwater Including Decision Trees on In Situ Bioremediation for Nitrates, Carbon Tetrachloride, and Perchlorate. (2002)
In Situ Bioremediation (ISB) guidance document addressing the systematic characterization, evaluation, and appropriate design and testing of ISB for biotreatable contaminants.

ITRC. In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones: Case Studies. (2007) (PDF) (138 pp, 2.3 MB)
Case studies on bioremediation of DNAPLs (BioDNAPLs) addressing the selection and design of in situ bioremediation (ISB) systems for chlorinated ethene DNAPL source zones, as well as technical and related regulatory considerations.

ITRC. In Situ Bioremediation of Chlorinated Ethene: DNAPL Source Zones. (2008a)
Bioremediation of DNAPLs (BioDNAPLs) guidance document addressing the selection and design of in situ bioremediation (ISB) systems for chlorinated ethene DNAPL source zones, as well as technical and related regulatory considerations.

ITRC. Enhanced Attenuation: Chlorinated Organics Guidance Documents Organics Document. (2008b)
Enhanced Attenuation (EA): Chlorinated Organics guidance documents provide direction to regulators and practitioners on integrating EA into remedial decision making for a smooth transition between aggressive remediation and monitored natural attenuation.

Lebrón, C., Wiedemeier, T. H., Wilson, J.T., Löffler, F.E., Hinchee, R.E., M. Singletary. Development and Validation of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites. ESTCP Project ER-201129. (2016)
The document and associated spreadsheet-based tool presents a systematic approach to evaluate whether MNA is an appropriate remedy based on site-specific conditions. The quantitative framework is a systematic decision-making protocol that allows the user to determine if degradation is occurring and, if it is, to deduce the relevant degradation pathway(s) based on the assessment of specific analytical parameters.

Lorenz, A. and Loffler, F. Organohalide-Respiring Bacteria. (2016).
The objective of this book is to provide a compendium of knowledge on organohalide-respiring microorganisms, their diversity and function as well as utility in degradation of chlorinated solvents.

NAVFAC. Design Consideration for Enhanced Reductive Dechlorination. (2015)
The purpose of this document is to provide a framework for design submittals for enhanced reductive dechlorination systems, including a summary of best practices for bioremediation design, tips for appropriate quality assurance and

quality control measures, and a listing of available standards and references. Lessons learned from Navy sites are shared related to the design, implementation, and performance of ERD for the remediation of chlorinated solvents in groundwater.

NAVFAC. Best Practices for Injection and Distribution of Amendments. Technical Report. (2013)
The objective of this document is to present current "best practices" for introducing liquid- and solid-phase amendments into aquifers and improve the likelihood that these amendments are adequately distributed. Best practices and lessons learned through evaluation of past applications of these technologies are provided.

NAVFAC Bioremediation-Using in Dense Non-Aqueous Phase Liquid Source Zones. Fact Sheet. (2012)
Under the right conditions, in situ bioremediation has been proven successful as a remedial strategy in chlorinated solvent source zones areas. This fact sheet summarizes initial screening factors that could help you to decide if ISB is right for your dense non-aqueous phase liquid (DNAPL) site.

NAVFAC. Bioremediation Overview. Web Tool. (2007a)
Biodegradation mechanisms are a cost-effective treatment option for sites where groundwater is contaminated with chlorinated solvents. This tool provides an overview of bioremediation approaches including monitored natural attenuation and enhanced anaerobic bioremediation.

NAVFAC. DCE/VC Stall. Web Tool. (2007b)
This tool discusses the common causes for DCE and VC stall and presents a variety of case studies to illustrate potential solutions. DCE and VC stall is an issue at several sites due to limitations preventing the complete dechlorination of the chlorinated ethenes to ethene. This tool discusses site limitations that may lead to DCE/VC stall and potential solutions including physical, biogeochemical, biostimulation, and bioaugmentation approaches.

NAVFAC. Molecular Biological Tools. Web Tool. (2007c)
This tool discusses a variety of molecular biological tools (MBTs). This tool provides an introduction to MBTs applied through gene-based, protein-based, lipid-based and isotope-based approaches.

NAVFAC. Assessing the Feasibility of DNAPL Source Zone Remediation: Review of Case Studies. (2004)
This report discusses developing guidelines for selecting dense, nonaqueous

phase liquid (DNAPL) source remediation guidelines by reviewing various DNAPL treatment technologies that have been field-deployed.

Stroo, H., A. Leeson and H. Ward. Bioaugmentation for Groundwater Remediation. (2013)
This reference book published by Springer reviews the past 10-15 years of research and development that have led to bioaugmentation becoming an effective and accepted remedial technology. Decision-making processes for implementing bioaugmentation, design and cost considerations, and monitoring options are described. The book serves as a reference for environmental remediation professionals seeking to understand, evaluate, and implement bioaugmentation.

Stroo, H. and Ward H. In Situ Remediation of Chlorinated Solvent Plumes. (2010)
This reference book published by Springer describes the process design and engineering for physical, chemical, and biological technologies used to treat complex chlorinated solvent plumes and is based largely on SERDP- and ESTCP-funded efforts. It serves as a reference for decision makers, practicing engineers, and hydrologists who select, design, and operate remedial systems, as well as researchers seeking to improve the state of the art.