Bioremediation

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Bioremediation uses microorganisms to degrade organic contaminants in soil, groundwater, sludge, and solids. The microorganisms break down contaminants by using them as an energy source or cometabolizing them with an energy source. More specifically, bioremediation involves the production of energy in a redox reaction within microbial cells. These reactions include respiration and other biological functions needed for cell maintenance and reproduction. A delivery system that provides one or more of the following is generally required: an energy source (electron donor), an electron acceptor, and nutrients. Different types of microbial electron acceptor classes can be involved in bioremediation, such as oxygen-, nitrate-, manganese-, iron (III)-, sulfate-, or carbon dioxide-reducing , and their corresponding redox potentials. Redox potentials provide an indication of the relative dominance of the electron acceptor classes (EPA 2000). Generally, electron acceptors and nutrients are the two most critical components of any delivery system (EPA 2004).

To stimulate and enhance microbial activity, microorganisms (**bioaugmentation**) or amendments (**biostimulation**), such as air, organic substrates or other electron donors/acceptors, nutrients, and other compounds that affect and can limit treatment in their absence can be added. Biostimulation can be used where the bacteria necessary to degrade the contaminants are present but conditions do not favor their growth (e.g., anaerobic bacteria in an aerobic aquifer, aerobic bacteria in an anaerobic aquifer, lack of appropriate nutrients or electron donors/acceptors). Bioaugumentation can be used when the bacteria necessary to degrade the contaminants do not occur naturally at a site or occur at too low of a population to be effective. Biostimulation and bioaugmentation can be used to treat soil and other solids, groundwater, or surface water (EPA 2006).

Under the proper conditions, monitored natural attenuation (MNA), which can include an intrinsic biodegradation process that depends on indigenous microorganisms to degrade contaminants without any amendments, may be an appropriate approach for a site.

Bioremediation may be conducted *in situ* or *ex situ*. In situ processes treat soil and groundwater in place, without removal or transportation offsite. This approach may be advantageous since the costs of materials handling and some environmental impacts may be reduced. However, *in situ* processes may be limited by the ability to control or manipulate the physical and chemical environment during bioremediation. *Ex situ* processes, on the other hand, involve the removal of the contaminated media to a treatment area (EPA 2006).

The first step of any bioremediation program is to develop a conceptual site model (CSM) to evaluate the potential for applying bioremediation at a site. The CSM takes into account the nature and extent of contamination and site characteristics; site hydrogeology, geochemistry and oxidation-reduction conditions; biodegradation potential; contaminant fate and transport; and receptor and exposure pathways. Once a CSM is established and refined, a characterization of the existing microbial community, or the characteristics necessary for the establishment of an appropriate microbial community, can be determined. Activities undertaken prior to the implementation of a bioremediation program often involve treatability studies, examination of soil comparability and the structure and function of the microbial community to ensure that undesirable reactions with the contaminants or their degradation products are prevented. The success of a bioremediation application highly depends on characterization and monitoring completed before and during its implementation (Hazen 2010).

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Types of Bioremediation

Aerobic bioremediation involves microbial reactions that require oxygen to go forward. The bacteria use a carbon substrate as the electron donor and oxygen as the electron acceptor. **Anaerobic bioremediation** involves microbial reactions occurring in the absence of oxygen and encompasses many processes, including fermentation, methanogenesis, reductive dechlorination, and sulfate- and nitrate reducing conditions. Depending on the contaminant, a subset of these activities may be cultivated. In anaerobic metabolism, nitrate, sulfate, carbon dioxide, oxidized materials, or organic compounds may replace oxygen as the electron acceptor. In **cometabolic bioremediation**, microbes do not gain energy or carbon from degrading a contaminant. Instead, the contaminant is degraded via a side reaction (EPA 2006).

The best bioremediation approach (aerobic, anaerobic, or cometabolic) largely depends on the type of contaminant(s) and site conditions present. The table below provides an overview of the biodegradability of numerous contaminants and the preferential (aerobic or anaerobic) conditions for degradation.

Contaminant Biodegradability and the Preferential Degradation Conditions

Contaminant	Microbial Degradability			Preferred Conditions
	High	*Low*	*No*	
Mineral oil hydrocarbons				
Short-chain mineral oil hydrocarbons	●			Aerobic
Long-chain/branched mineral oil hydrocarbons		●		Aerobic
Cycloalkanes		●		Aerobic
Monoaromatic hydrocarbons				
(Mono)aromatic hydrocarbons	●			Aerobic
Phenols	●			Aerobic
Cresols		●		Aerobic
Catechols	●			Aerobic
Polycyclic Aromatic Hydrocarbons				
2- to 3-ring-PAHs (e.g., naphthalene)	●			Aerobic
4- to 6-membered ring PAHs (e.g.,benzo(a)pyrene)				

		●		Aerobic
Chlorinated Aliphatic Hydrocarbons				
Tetrachloroethene, trichloroethane	●			Anaerobic
Trichloroethene	●			Anaerobic
Dichloroethane, dichloroethene, vinyl chloride	●			Anaerobic/aerobic
Chlorinated Aromatic Hydrocarbons				
Chlorophenols (superchlorinated)		●		Anaerobic
Chlorophenols (low-chlorinated)	●			Anaerobic/aerobic
Chlorobenzenes (superchlorinated)		●		Anaerobic
Chlorbenzenes (low-chlorinated)	●			Anaerobic/aerobic
Chloronaphthalene	●			Anaerobic/aerobic
* Polychlorinated biphenyls (PCBs) (superchlorinated)		●		Anaerobic
Polychlorinated biphenyls (low-chlorinated)	●			Anaerobic/aerobic
Nitroaromatic Compounds				
Mono- and dinitroaromatics	●			Anaerobic/aerobic
Trinitrotoluene (TNT)	●			Anaerobic/aerobic
Trinitrophenol (picric acid)		●		Anaerobic/aerobic
Nitroaliphatic Compounds				
Glycerol trinitrate	●			Aerobic
Pesticides				
g-hexachlorocyclohexane (lindane)	●			Anaerobic/aerobic
b-hexachlorocyclohexane (lindane)		** ●	●	Anaerobic/aerobic
Atrazines				Aerobic

	●			
Dioxins				
PCDD/F (several)		●		Anaerobic
2,3,7,8-PCDD/PCDF			●	
Inorganic Compounds				
Free cyanides		●		Aerobic
Complex cyanides		●		
Ammonium	●			Anaerobic/aerobic
Nitrate	●			Anaerobic
***Sulfate	●			Anaerobic

* The degradation process and susceptibility to degradation are different for planar and non-planar highly chlorinated PCB congeners

** Microbially transformable but not degradable

***Activity of sulfate-reducing bacteria results in precipitation of metal sulfides or production of hydrogen sulfide gas

Adapted from the International Centre for Soil and Contaminated Sites (ICSS)Manual for Biological Remediation Techniques (2006)

The CLU-IN focus page on DNAPLs provides additional information on bioremediation approaches and preferred conditions (aerobic or anaerobic) for numerous **halogenated alkanes** and **halogenated alkenes**, including ethanes and ethenes.

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Factors that Affect Bioremediation

Contaminant concentrations directly influence microbial activity. When concentrations are too high, the contaminants may have toxic effects on the present bacteria. In contrast, low contaminant concentration may prevent induction of bacterial degradation enzymes.

Contaminant bioavailability depends on the degree to which they sorb to solids or are sequestered by molecules in contaminated media, are diffused in macropores of soil or sediment, and other factors such as whether contaminants are present in non-aqueous phase liquid (NAPL) form. Bioavailability for microbial reactions is lower for contaminants that are more strongly sorbed to solids, enclosed in matrices of molecules in contaminated media, more widely diffused in macropores of soil and sediments, or are present in NAPL form (ICSS 2006).

Site characteristics have a significant impact on the effectiveness of any bioremediation strategy. Site environmental conditions important to consider for bioremediation applications include pH, temperature, water content, nutrient availability, and redox potential.

pH affects the solubility and biological availability of nutrients, metals, and other constituents; for optimal bacterial growth, pH should remain within the tolerance range for the target microorganisms (ESTCP 2005). Bioremediation processes preferentially proceed at a pH of 6-8 (ICSS 2006).

Redox Potential and oxygen content typify oxidizing or reducing conditions. Redox potential is influenced by the presence of electron acceptors such as nitrate, manganese oxides, iron oxides and sulfate (ICSS 2006).

Nutrients are needed for microbial cell growth and division (ESTCP 2005). Suitable amounts of trace nutrients for microbial growth are usually present, but nutrients can be added in a useable form or via an organic substrate amendment (Parsons 2004), which also serves as an electron donor, to stimulate bioremediation.

Temperature directly affects the rate of microbial metabolism and consequently microbial activity in the environment. The biodegradation rate, to an extent rises with increasing temperature and slows with decreasing temperature (ESTCP 2005).

References:

Environmental Security Technology Certification Program (ESTCP) 2005. Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development Status and Research Needs.

This report summarizes the technical and regulatory status of bioaugmentation for chlorinated ethenes and identifies research needs to be addressed to facilitate successful widespread use of the technology.

EPA 2000. Engineered Approaches to In Situ Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications EPA 542-R-00-008.

EPA 2004. In-Situ Groundwater Bioremediation. Chapter 10 in How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Sites: A Guide for Corrective Action Plan Reviewers. EPA 510-R-04-002.

EPA 2006 Engineering Issue: In Situ and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites. EPA-625-R-06-015.

Hazen, T.C. 2010. In Situ Groundwater Bioremediation. Chapter 13 in Part 24 of the Handbook of Hydrocarbon and Lipid Microbiology. Springer-Verlag Berlin Heidelberg, ISBN: 978-3-540-77587-4, p 2584-2596.

This paper provides an overview of bioremediation concepts involving intrinsic biodegradation, biostimulation, and bioaugmentation for a variety of contaminants, including chlorinated hydrocarbons.

International Centre for Soil and Contaminated Sites (ICSS) 2006. Manual for Biological Remediation Techniques.

Parsons. 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. AFCEE, NFEC, ESTCP 457 pp, August 2004.

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Additional Information

Aerobic Biodegradation of Oily Wastes: A Field Guidance Book for Federal On-Scene Coordinators
Version 1.0, October 2003
EPA Region 6

The objective of this field guide is to provide guidance (primarily to federal On-Scene Coordinators) in selecting and conducting land aerobic biodegradation of oil-contaminated wastes from inland oil spills, leaking/unplugged oil wells, abandoned oil refinery sites, pipeline ruptures, and tank failures. The first part of the field guide provides information to help evaluate the nature of the environment where land treatment is considered and a summary of the existing regulations and policies (in EPA Region 6). The second part provides an overview of the factors to be considered and studied when determining if land farming is a viable option and also discusses key points in the process design. The last part focuses on operation issues and provides useful tools and information for efficient management of aerobic land treatments.

Applicability of RCRA Section 3020 to In-Situ Treatment of Ground Water

2000

This memorandum clarifies that reinjection of treated groundwater to promote in situ treatment is allowed under section 3020(b) as long as certain conditions are met. Specifically, the groundwater must be treated prior to reinjection; the treatment must be intended to substantially reduce hazardous constituents in the groundwater – either before or after reinjection; the cleanup must be protective of human health and the environment; and the injection must be part of a response action under CERCLA section 104 or 106 or a RCRA corrective action intended to clean up the contamination.

Applications and Benefits of Groundwater Recirculation for Electron Donor Delivery and pH-Adjustment during Enhanced Anaerobic Dechlorination

Falatko, D.M., S.A. Fam, and G. Pon.

Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy 16:77-89(2011)

The proper design and implementation of groundwater recirculation for in situ enhanced anaerobic dechlorination of various chlorinated organic compounds is presented with a review of the applicable concepts.

Attachment A: Conceptual Site Model Summary

1996

The Conceptual Site Model summary forms and worksheets contain the information necessary to determine the applicability of soil screening levels (SSLs) to the site, and help focus data collection efforts to gather information needed to calculate SSLs.

Bioaugmentation for Groundwater Remediation

Stroo, H.F., A. Leeson, and C.H. Ward (eds).

Springer, New York . SERDP-ESTCP Environmental Remediation Technology, Vol 5. ISBN: 978-1-4614-4114-4, 389 pp, 2013

This volume offers a review of the past 10 to 15 years of intensive research and development that has led to the acceptance of bioaugmentation technology. It provides background information on the basic microbial processes involved and a summary of the most important bioaugmentation strategies. In addition to production and handling of Dehalococcoides bioaugmentation cultures, the text covers bioaugmentation for MTBE remediation, carbon tetrachloride remediation, aerobic degradation of DCE, and in situ aerobic cometabolism of chlorinated solvents. Table of contents with abstracts

Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs

Environmental Security Technology Certification Program (ESTCP). 126 pp, Oct 2005

This white paper reviews the state of bioaugmentation science at the present time, summarizes the current status of this rapidly evolving innovative technology, identifies the key issues confronting the science, and evaluates the lessons learned from current practical applications. This technology 'snapshot' may be useful to remedial project managers faced with selecting, designing, and implementing a bioaugmentation strategy.

Biocatalysis/Biodegradation Database (University of Minnesota)

This database contains information on microbial biocatalytic reactions and biodegradation pathways for primarily xenobiotic (chemical substance foreign to an organism or biological system) chemical compounds. The goal of the University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD) is to provide information on microbial enzyme-catalyzed reactions that are important for biotechnology.

Biocell Technology: Remediation of Petroleum-Contaminated Soils

1998

This technology data sheet describes biocell (bioreactor) technology and provides information on the research on and demonstration of this technology at the Army's Waterways Experiment Station, where petroleum-contaminated soils were loaded into a 10 yd^3

biocell. Aerobic microbial activity was stimulated within the soils through aeration.

Figure 1. The 10-yd^3 biocell at Port Hueneme, California

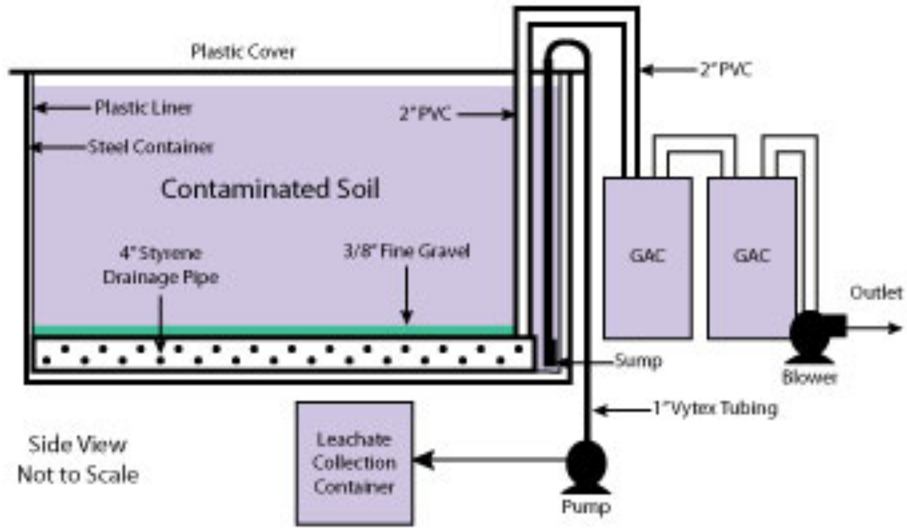

Figure 2. Schematic of a 40-yd^3 biocell.

A Citizen's Guide to Bioremediation
EPA 542-F-12-003, 2012

The Citizen's Guide series summarize cleanup methods used at Superfund and other sites. Each two-page fact sheet answers six questions about the cleanup method: 1) What is it? 2) How does it work? 3) How long will it take? 4) Is it safe? 5) How might it affect me? and 6) Why use it?

Contaminants in the Subsurface: Source Zone Assessment and Remediation
National Research Council, Committee on Source Removal of Contaminants in the Subsurface. National Academies Press, Washington, DC. ISBN: 030909447X, 383 pp, 2004

After discussing the definition of 'source zone' and the characterization thereof, this report reviews the suite of technologies available for source remediation and their ability to reach a variety of cleanup goals, from meeting regulatory standards for groundwater to reducing costs. The report proposes elements of a protocol for accomplishing source remediation that should enable project managers to decide whether and how to pursue source remediation their sites.

Cornell University Waste Management Institute (Composting)

This Resource Page contains a multitude of resources on small- and large-scale composting.

Cost and Performance Report for Bioavailable Ferric Iron (BAFeIII) Assay
Environmental Security Technology Certification Program, ESTCP Project ER-0009, 43 pp, Feb 2007

This report describes the demonstration and validation at four DoD installations of a bioavailable ferric iron (BAFe[III]) assay. BAFe(III) is defined as ferric iron (Fe[III]), a form that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer. BAFe(III) is an important terminal electron acceptor with significant assimilative capacity in many natural environments. The overall objective of this project was to demonstrate and validate the performance of the BAFe(III) assay as an analytical technology for use in supporting bioremediation. Specific objectives were to validate the BAFe(III) assay method using a combination of confirmatory analyses and to quantify costs associated with the technology.

Dense Nonaqueous Phase Liquids (DNAPLs) Treatment Technologies Bioremediation

This CLU-IN Web page provides a discussion of bioremediation techniques for DNAPL chemicals, most of which are biologically degraded under anaerobic conditions. Included are citations and case studies.

Development and Validation of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches at Chlorinated Ethene Sites
Lebron, C., T. Wiedemeier, J. Wilson, F. Loeffler, R. Hinchee, and M. Singletary.
ESTCP Project ER-201129, 178 pp, 2015

The overarching project objective was to develop and validate a framework for making bioremediation decisions based on site-specific physical and biogeochemical characteristics and constraints. The key deliverable is called BioPIC, an easy-to-use decision tool for estimating and integrating the impact of quantifiable parameters on NA and microbial remedies to achieve detoxification of chlorinated ethenes. The quantitative framework and BioPIC were beta-tested for chlorinated ethenes (mainly PCE, TCE, and daughter products) degradation at four sites. Additional information: BioPIC tool and other reports

Development of Assessment Tools for Evaluation of the Benefits of DNAPL Source Zone Treatment
Abriola, L.M., P. Goovaerts, K.D. Pennell, and F.E. Loeffler.
SERDP Project ER-1293, 173 pp, 2008

This report details the results of work that has enhanced the understanding of significant mechanisms controlling DNAPL source zone behavior and describes lessons learned that can provide improved DNAPL site management strategies. It discusses 4 important concepts: (1) partial source-zone mass removal can result in substantial local concentration and mass flux reductions; (2) potential remediation efficiency is closely linked to source-zone architecture (ganglia-to-pool ratios); (3) biostimulation and bioaugmentation approaches are feasible for treatment of DNAPL source zones; and (4) the uncertainty in mass discharge ([M/T]) estimates can be quantified through application of geostatistical methods to field measurements.

Development of Bioreactors for Application of Biocatalysts in Biotransformations and Bioremediation
2001

This paper summarizes research on application of biofilms of fungal and bacterial cells and their enzymes, including hydrolases, polyphenol oxidase, peroxidase and laccase, in bioreactor systems including continuously operating membrane bioreactors.

Development of a Design Tool for Planning Aqueous Amendment Injection Systems: User's Guide
Borden, R.C. et al.
ESTCP Project ER-0626

A simple spreadsheet-based tool developed to assist in the design of injection-only systems for distributing emulsions or soluble substrate allows quick comparison of the relative costs and performance of different injection alternatives and identification of the design best suited to site-specific conditions. Emulsion Design Tool (2008); Soluble Substrate Design Tool (2012).

Draft Technical Protocol: A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) to Remediate Chloroethenes
Morse; J.J., B.C. Alleman; J.M. Gossett; S.H. Zinder; D.E. Fennell, Battelle Memorial Inst., Columbus, OH. AFRL-ML-TY-TR-1998-4522, NTIS: ADA352416/XAB, 94 pp, 1998.

This document describes a comprehensive approach for conducting a phased treatability test to determine the potential for employing RABITT at any specific site. It is not meant as a guide for designing either full or pilot-scale in situ biotreatment systems for chlorinated ethenes or any other contaminant. The protocol guides the user through a decision process in which information is collected and evaluated to determine if the technology should be given further consideration. RABITT will be screened out if it is determined that site-specific characteristics, regulatory constraints, or other logistic problems suggest that the technology will be difficult or impossible to employ, or if a competing technology clearly is superior.

Elucidation of the Mechanisms and Environmental Relevance of cis-Dichloroethene and Vinyl Chloride Biodegradation
Cox, E.
SERDP Project ER-1557, 170 pp, 2012

Major results of this project can be summarized as follows: (1) JS666 remains the only isolated organism known to mediate aerobic oxidation of cDCE to CO_2, and DNA-based molecular biological tools exist to track its presence and fate during bioaugmentation projects; (2) significant advances were made in understanding the pathway, mechanisms, and enzymes associated with aerobic oxidation of cDCE in JS666; (3) anaerobic oxidation of cDCE and/or VC under iron- or manganese-reducing conditions could not be confirmed, despite substantial efforts with materials from many sites; (4) suspected anaerobic oxidation of VC may in fact be aerobic oxidation to CO_2 at extremely low levels of oxygen in the subsurface; and (5) compound-specific isotope fractionation of carbon occurs in both anaerobic and aerobic microbial degradation of ethane, allowing the use of CSIA to assess ethene degradation as a possible means to explain poor ethene mass balance in enhanced in situ bioremediation and MNA projects.

Enhanced Attenuation: A Reference Guide on Approaches to Increase the Natural Treatment Capacity of a System
Early, T., B. Borden, M. Heitkamp, B.B. Looney, D. Major, W.J. Waugh, G. Wein, T. Wiedemeier, K.M. Vangelas, K.M. Adams, and C.H. Sink. WSRC-STI-2006-00083, Revision 1, 161 pp, Aug 2006

This guide covers the following EA approaches: (1) hydraulic manipulation to reduce contaminant infiltration using low-permeability barriers, diffusion barriers, covers, encapsulation, and diversion of electron acceptors; (2) passive residual source reduction (e.g., bioventing); (3) increase in system attenuation capacity via biological processes, such as bioaugmentation, biostimulation, and wetlands development and other plant-based methods; (4) abiotic and biologically mediated abiotic attenuation methods; and (5) reactive barriers.

Environmental Molecular Diagnostics: New Site Characterization and Remediation Enhancement Tools
Interstate Technology & Regulatory Council (ITRC), Environmental Molecular Diagnostics Team. EMD-2, 363 pp, Apr 2013

> EMD technologies can be classified into two major categories of analytical techniques: chemical technologies (i.e., CSIA), and different molecular biological techniques. A detailed description of each major EMD is illustrated with case studies of their application and recommendations regarding appropriate uses. Frequently asked questions regarding the underlying science, including stable isotope chemistry and fundamental molecular biology, are addressed in the appendices. Also available as a PDF file

Enzyme Activity Probe and Geochemical Assessment for Potential Aerobic Cometabolism of Trichloroethene in Groundwater of the Northwest Plume, Paducah Gaseous Diffusion Plant, Kentucky
Office of Environmental Management
DOE, WSRC-STI-2008-00309, 88 pp, 2008

Exploitation of Composting Management for Either Reclamation of Organic Wastes or Solid-Phase Treatment of Contaminated Environmental Matrices
2002

> This paper provides an overview of the potential use of composting technology in programs aimed at organic waste recycling (product-oriented perspective) or decomposition of hazardous materials.

Feasibility of Calcium Peroxide as an Oxygen Releasing Compound in Treatment Walls
2008

> This paper investigates the use of a proprietary formulation of powdered calcium peroxide as an oxygen releasing compound in a treatment wall. Laboratory-scale column studies evaluated the release of oxygen and the permeability effects resulting from a treatment wall mixture of the calcium peroxide and representative aquifer sand. The research focused on measuring permeability effects within the treatment wall due to the initial addition and subsequent chemical reduction of the calcium peroxide and the degree to which dissolved oxygen concentration increased in water flowing out of the treatment wall.

Field Push-Pull Test Protocol for Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons
Kim, Y., M. Azizian, J. Istok, and L. Semprini.
Environmental Security Technology Certification Program, 83 pp, 2005

> This protocol describes a single-well push-pull test for evaluating the feasibility of using in situ aerobic cometabolic processes to treat groundwater contaminated with chlorinated solvent mixtures.

Green Remediation Best Management Practices: Bioremediation
EPA 542-F-10-006, 2010

> The U.S. Environmental Protection Agency (EPA) Principles for Greener Cleanups outlines the Agency's policy for evaluating and minimizing the environmental 'footprint' of activities undertaken when cleaning up a contaminated site. Use of the best management practices (BMPs) recommended in EPA's series of green remediation fact sheets can help project managers and other stakeholders apply the principles on a routine basis, while maintaining the cleanup objectives, ensuring protectiveness of a remedy, and improving its environmental outcome. Bioremediation actively enhances the effects of naturally occurring biological processes that degrade contaminants in soil, sediment, and groundwater.

Guidance Protocol: Application of Nucleic Acid-Based Tools for Monitoring Monitored Natural Attenuation (MNA), Biostimulation, and Bioaugmentation at Chlorinated Solvent Sites
ESTCP Project ER-0518, 34 pp, 2011

> This protocol summarizes the current state of the practice of molecular biological tools (MBTs), specifically nucleic-acid based tools commercially available to identify relevant *Dehalococcoides* bacteria. It is intended to provide a technically sound and practical approach to MBT use. This document provides recommendations regarding sampling approaches and criteria in evaluation of data for use in bioremediation decision making. See also the Project ER-0518 Final Report and the ESTCP Cost and Performance Report.

IRP Aerobic Cometabolic In Situ Bioremediation Technology Guidance Manual and Screening Software User's Guide
Earth Technology Corp., Alexandria, VA Report No: AFRL-ML-TY-TR-1998-4530. NTIS Order No: ADA359333/XAB. 84 pp, June 1998

This document presents the principles of the process, mathematical models used to describe the aerobic cometabolic in situ bioremediation technology, and a discussion of its applicability and limitations. A description is also provided of a software program that can help determine if this technology is appropriate for implementation. The technology was implemented in a full-scale evaluation at Edwards AFB, California. The document includes a discussion surrounding regulatory acceptance of the technology and a description of other field implementations. The report is designed for use by project managers who are exploring potential technology alternatives for groundwater treatment under the Installation Restoration Program (IRP).

In Situ Bioremediation of Chlorinated Ethene: DNAPL Source Zones
Interstate Technology & Regulatory Council (ITRC), Bioremediation of DNAPLs Team. BioDNAPL-3, 138 pp, June 2008

This publication systematically lays out the technical and related regulatory considerations for in situ bioremediation (ISB) of chlorinated ethene DNAPL source zones, providing information related to site characterization requirements, treatment system application and design criteria, process monitoring, and process optimization. The ability of ISB technology to enhance the dissolution and desorption of nonaqueous-phase contaminants to the aqueous phase, where they can be degraded by the microbial population, depends on the spatial distribution of DNAPL mass in the subsurface (e.g., pool/ganglia ratio) and the ability to deliver amendments throughout this architecture.

In Situ Bioremediation of DNAPL Source Zones
2005

This document was prepared by Lisa Moretti, a National Network of Environmental Management studies grantee, under a fellowship from the U.S. Environmental Protection Agency. The objective of this report is to provide an overview of in situ bioremediation of DNAPL source areas. This report discusses the integral steps when implementing bioremediation, such as site characterization, design considerations, and post-treatment monitoring. In addition, this report also examines the use of bioremediation as a polishing treatment for the source zone. Case studies are included as examples of the use of bioremediation as a stand-alone and a polishing treatment for DNAPL source areas. This report was not subject to EPA peer review or technical review. EPA makes no warranties, expressed or implied, including without limitation, warranties for completeness, accuracy, usefulness of the information, merchantability, or fitness for a particular purpose.

In Situ Bioremediation of TCE-Contaminated Groundwater
Travis, B.J. (Los Alamos National Lab., NM); N.D. Rosenberg (Lawrence Livermore National Lab., CA). LA-UR-98-2605, NTIS: DE99001639, 22 pp, 1998

The authors have developed a biokinetics model that includes microbial competition and predation processes. Predator species can feed on the microbial species that degrade contaminants. Simulation studies show that species interactions must be considered when designing in situ bioremediation systems. This report is the final product of a two-year, Laboratory Directed Research and Development (LDRD) project the Los Alamos National Laboratory. The report is available through the DOE Information Bridge.

In-Situ Bioremediation of Chlorinated Hydrocarbons: An Assessment of Projects in California
California Department of Toxic Substances Control, Office of Pollution Prevention and Technology Development. OPPTD Document No. 1217, 163 pp, 2006.

During an evaluation of the performance of in situ bioremediation (ISB) systems at 5 sites in California, the reviewers observed several recurring issues. The project case studies illustrate the reviewers' recommendations for avoiding common ISB problems.

In-Situ Substrate Addition to Create Reactive Zones for Treatment of Chlorinated Aliphatic Hydrocarbons: ESTCP Cost and Performance Report
Environmental Security Technology Certification Program (ESTCP), CU-9920, 93 pp, Mar 2007.

Demonstrations of enhanced reductive dechlorination (ERD) were conducted at two Air Force bases—Vandenberg and Hanscom—to show the ability of this bioremediation approach to dechlorinate TCE plumes in the subsurface over a relatively short time period and to gather information for estimating long-term treatment effectiveness, life span, and costs.

Introduction to In Situ Bioremediation of Groundwater
EPA 542-R-13-018, 2013

Introduction to In Situ Bioremediation of Groundwater was prepared by the Office of Superfund Remediation and Technology Innovation (OSRTI) as an introduction to in situ bioremediation of groundwater. This information is intended for U.S. Environmental Protection Agency and state agency site managers and may serve as a reference to designers and practitioners.

Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation
Henry, B.
ESTCP Project ER-0627, 476 pp, 2010

The author evaluated 15 case studies of different substrates used to stimulate biodegradation of chlorinated compounds: Hydrogen Release Compounds (HRC and HRC-X), vegetable oil (neat and emulsified), whey, molasses, ethanol and lactate, and mulch in permeable biowalls. This report discusses the factors that limit enhanced in situ bioremediation and describes (in Appendix B) a Substrate Design Tool developed in Microsoft Excel to assist the practitioner in evaluating a site for an application of enhanced in situ bioremediation. Substrate Design Tool; ESTCP Cost & Performance Report; 2010 Addendum

Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation: Addendum to the Principles and Practices Manual
Henry, B.
Environmental Security Technology Certification Program (ESTCP), Project ER-200627, 39 pp, Jan 2010

Improvements and advances in the enhanced in situ bioremediation of chlorinated solvents have been made since the Tri-Services released Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents in August 2004. This addendum to the 2004 document provides a description of a demonstration study conducted to evaluate substrate loading rates, including a summary of limiting factors and challenges to applying enhanced in situ bioremediation. The demonstration study involved the evaluation of 15 case studies for system design, operation, and performance. Quantitative and qualitative performance objectives were developed to evaluate the case studies and to identify limiting factors for enhanced in situ bioremediation. Supporting information for the case studies can be found in the 2010 Final Technology Demonstration Report. This addendum also summarizes advances made in the field of enhanced in situ bioremediation of chlorinated solvents over the last six years and provides resources and references that can be used to identify and mitigate the limiting factors and challenges that practitioners face when applying the technology.

Manual for Biological Remediation Techniques
International Centre for Soil and Contaminated Sites, 81 pp, 2006

Provides an initial overview of selected organic contaminants, describes their susceptibility to microbial degradation in soil and groundwater, and reviews their treatment potential by land farming, biobeds, bioreactors, bioslurping, bioventing, biosparging, bioscreen, bioaugmentation, and monitored natural attenuation. Monitoring of bioremediation progress is also discussed.

Natural Attenuation and Biodegradation of Contaminants
US Geological Survey Toxic Substances Hydrology Program Bibliography

Provides a bibliographic reference for resources related to bioremediation, including scientific journal publications, conference presentations, agency reports, and others.

Natural Attenuation of Chlorinated Solvents in Groundwater: Principles and Practices
1999

This Principles and Practices Document was prepared by the Industrial Members of the Bioremediation of Chlorinated Solvents Consortium of the Remediation Technologies Development Forum (RTDF). The document provides a description of practices to be used to recognize and evaluate the presence of natural attenuation of chlorinated solvent contamination.

Operation and Analysis of the BEHIVS System at Edwards Air Force Base
McCarty, P.L., S.M. Gorelick, M.N. Goltz, G.D. Hopkins, and F.-J. Eisenberg.
Strategic Environmental Research and Development Program (SERDP). 109 pp, 2003

This report summarizes the results of operation of the bioenhanced in-well vapor stripping (BEHIVS) system at Edwards AFB in 2001, numerical modeling analysis of the results, study conclusions, and recommendations for application of the BEHIVS system at other sites.

Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones
The Interstate Technology & Regulatory Council (ITRC) Bioremediation of DNAPLs Team.
BioDNAPL-1, 89 pp, 2005.

This document presents a technological overview of in situ bioremediation and some of the issues to consider when selecting and designing an in situ bioremediation system for remediation of chlorinated ethene DNAPL source zones. The document provides an overview of chlorinated ethene DNAPLs and in situ bioremediation, technical considerations for in situ bioremediation of chlorinated ethene DNAPL source zones, the state of in situ bioremediation technology applications, and information on defining and measuring system performance of in situ bioremediation applications for chlorinated ethene DNAPL sources.

Perchlorate: Overview of Issues, Status, and Remedial Options
Interstate Technology and Regulatory Council, 2005.

This document provides an overview of the commercially available technologies (including bioremediation) and emerging technologies that were still at the bench or pilot-scale stage at the time of publication.

Petroleum Bioventing
van Eyk, J. and A.A. Balkema, Rotterdam ; Brookfield, VT. ISBN: 9054106867. 302 pp, 1997

This book investigates the composition and the behavior of petroleum in soil, soil properties and soil processes, their interaction with bacterial processes, possibilities for optimizing the removal of petroleum hydrocarbons from soil by bacteria and it explains the phenomenon of recalcitrance.

Principles and Practices of Bioventing
Leeson, A. and R. Hinchee Battelle Memorial Institute. EPA 540-R-95-534a & b [2 vols.] 1995

The manual provides details on bioventing principles; site characterization; field treatability studies; system design, installation, and operation; process monitoring; site closure; and optional technologies to combine with bioventing if warranted. Volume 1 describes the basic principles of bioventing. Volume 2 focuses on bioventing design and process monitoring.

- **Volume 1 (4.8MB/88pp/PDF)**
- **Volume 2 (848KB/89pp/PDF)**

Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents
Parsons
AFCEE, 457 pp, 2004

This document was published by AFCEE, NFESC, and ESTCP to describe the state of the practice of enhanced anaerobic bioremediation. The text explains the scientific basis of enhanced anaerobic bioremediation and discusses relevant site selection, design, and performance criteria for various engineered approaches in current practice. The information is intended to help restoration or remedial project managers make informed decisions about enhanced bioremediation as a remedial alternative, select specific enhanced bioremediation approaches that are suitable for achieving remedial goals, and track the cost and performance of enhanced bioremediation applications. 2010 Addendum: Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation

Procedures for Conducting Bioventing Pilot Tests and Long-Term Monitoring of Bioventing Systems
Downey, D., R. Miller, & T. Dragoo, Parsons Denver, CO. NTIS: ADA423587, 80 pp 2004

This report replaces AFCEE's 1992 'Test Plan and Technical Protocol for a Field Treatability Test for Bioventing' and identifies an updated approach for conducting bioventing pilot tests and monitoring the long-term progress of bioventing systems.

Push-Pull Tests for Evaluating the Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons: ESTCP Cost and Performance Report
Environmental Security Technology Certification Program, NTIS: ADA468544, 46 pp, 2006

Single-well push/pull test methods were demonstrated at Fort Lewis Logistics Center (using toluene as a cometabolic growth substrate) and McClellan AFB (during cometabolic air sparging with propane as a growth substrate) to determine (1) the transport characteristics of nutrients, substrates, and CAHs and their transformation products; (2) the capability of indigenous microorganisms to utilize selected substrates and transform targeted contaminants and surrogate compounds; (3) the rates of substrate utilization and contaminant transformation; and (4) the combinations of injected nutrients and substrates that maximize rates of contaminant transformation.

Reductive Anaerobic Biological In-Situ Treatment Technology (RABITT) Treatability Test. Interim Report
Environmental Security Technology Certification Program (ESTCP), Arlington, VA, 16 pp, 2001.

This document presents a summary of the reductive anaerobic biological in situ treatment technology (RABITT) protocol. It also provides the results of treatability tests (both field and microcosm studies) of the RABITT protocol at Cape Canaveral Air Station, FL, Naval Air Station Alameda, CA, Ft. Lewis, WA, and Marine Corps Base, Camp Lejeune, NC.

Remediation Technologies for Perchlorate Contamination in Water and Soil
Interstate Technology and Regulatory Council, 2008.

This document provides an overview of perchlorate issues, site evaluation issues, considerations for the selection of a particular remedy, regulatory considerations, physical processes for treatment of perchlorate-impacted water, in situ and ex situ bioremediation technologies for perchlorate in water, remediation technologies for soil, and stakeholder issues.

A Review of Biofouling Controls for Enhanced In Situ Bioremediation of Groundwater

Environmental Security & Technology Certification Program (ESTCP), Project ER-0429, 55 pp, 2005

The objective of this report is to review well rehabilitation and biofouling controls that are potentially relevant to enhanced in situ bioremediation applications and to identify promising biofouling controls for comparative field evaluation and validation under Environmental Security and Technology Certification Program (ESTCP) Project ER-0429. The report presents a summary of biofouling causes and mechanisms; a discussion of the differences between well rehabilitation and preventative biofouling control, including a review of case studies where biofouling controls have been used in groundwater remediation applications; identification, evaluation and scoring of promising biofouling control options for further field evaluation/validation; and conclusions for preventive biofouling controls.

Soil Bioventing: Principles and Practice

Leeson; A., R.E. Hinchee, and et al. 1997. CRC/Lewis Publishers, Boca Raton, FL.

This book explains in practical terms how to carry out a bioventing program. The book discusses physical and microbial processes affecting bioventing, site characterization activities for implementation of bioventing, system design, performance monitoring, and process evaluation. Case histories of early bioventing studies are discussed as well.

Standardized Procedures for Use of Nucleic Acid-Based Tools: Recommendations for Groundwater Sampling and Analysis Using qPCR

Lebron, C., P. Dennis, C. Acheson, N. Barros, D. Major, E. Petrovskis, F. Loeffler, K. Ritalahti, C. Yeager, E. Edwards, J. Hatt, and D. Ogles. SERDP Project ER-1561, 12 pp, 2014

SERDP project ER-1561 focused on identifying and minimizing the causes of variability during quantitative real-time polymerase chain reaction (qPCR) enumeration of genes of interest in groundwater, with the goal of developing of the knowledge needed to standardize methods for collecting, preserving, transporting, storing, and processing environmental samples for qPCR analysis. This document summarizes the project conclusions and recommends procedures for using qPCR analyses that will provide data of sufficient accuracy and reproducibility to allow site management decisions regarding bioremediation or MNA. Further details are available in the ER-1561 Final Report (Lebron et al. 2014, 220 pages).

Strategies for Monitoring the Performance of DNAPL Source Zone Remedies

Interstate Technology and Regulatory Council (ITRC) Dense Nonaqueous-Phase Liquids Team. DNAPLs-5, 206 pp., Aug 2004

This document is intended for regulators and others interested in learning about approaches to performance monitoring while implementing various in situ technologies for the treatment of DNAPLs. In this document, we present a number of ways in which the success or failure in treating a DNAPL source zone has been measured. Because the vast majority of experience in DNAPL source zone remediation has been in unconsolidated geologies, such as sands and silts, many of the conclusions, recommendations, and lessons learned presented in this document do not necessarily transfer to performance assessment in fractured bedrock, karst, or other consolidated geologies.

A Summary of the DOE/PERF Bioremediation Workshop May 30, 2002 Houston, Texas

DOE, 25 pp, 2002

This document is a summary of a joint bioremediation workshop held in Houston, Texas on May 30, 2002, by the United States Department of Energy and the Petroleum Environmental Research Forum. The main objective of the workshop was to discuss the "state of the art" of bioremediation for hydrocarbon-impacted soil. Key findings from bioremediation research on marine, freshwater, and wetland oil spills were presented. Presentations at the workshop addressed bioremediation as practiced by the oil industry, toxicity assessment after bioremediation, and other technical issues. This workshop summary has been written in a "Question and Answer" format in order to provide the information in a concise manner for environmental professionals who are considering the use of bioremediation at sites where hydrocarbons have impacted soils.

Superfund Remedy Report, Fourteenth Edition

2013

The Superfund Remedy Report (SRR), Fourteenth Edition, was published by the EPA Office of Superfund Remediation and Technology Innovation (OSRTI) in November 2013. The SRR 14th Edition summarizes remedy decisions back to 1982 with a focus on the analysis of Superfund remedial actions selected from fiscal years (FY) 2009 to 2011. The report includes remedies selected in 459 decision documents (Records of Decision [RODs], ROD amendments, and Explanations of Significant Differences with changes to remedy components) signed in this three-year period. The SRR compiles data on overall remedy selection and remedies for source materials (such as soil and sediments), groundwater, surface water and air related to vapor intrusion. This edition of the report, for the first time, presents a detailed look at sediment remedies and an analysis of vapor intrusion remedies. The report also analyzes media and contaminants for sites under investigation with planned RODs. The online version includes downloadable appendices that summarize all the remedy components selected for sources and groundwater in each individual decision document.

- **View or Download Previous Editions**

A Systematic Approach to In Situ Bioremediation in Groundwater, Including Decision Trees for In Situ Bioremediation of Nitrates, Carbon Tetrachloride, and Perchlorate
Interstate Technology and Regulatory Council. ITRC ISB-8, 158 pp, 2002

This document provides guidance for the systematic characterization, evaluation, and appropriate design and testing of in situ bioremediation for any biotreatable contaminant. It includes information on considerations for a bioremediation program, including site background, hydrogeology/geochemistry, contaminant fate and transport, and limitations. The document also provides information on systematic approaches to in situ bioremediation of nitrate, carbon tetrachloride, and perchlorate.

Using Chemical Priming as a Means of Enhancing the Performance of Biocells for Treating Petroleum Products Containing Recalcitrant Chemical Species
Wang, Winnie, et al., Mississippi State University, 158 pp, 2001

Biocell technology is a soil remediation technology that utilizes commercial roll-off dumpsters as simple, yet effective bioreactors. This report evaluates the effectiveness of using chemical oxidizers to aid in the bioremediation of petroleum hydrocarbons.

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