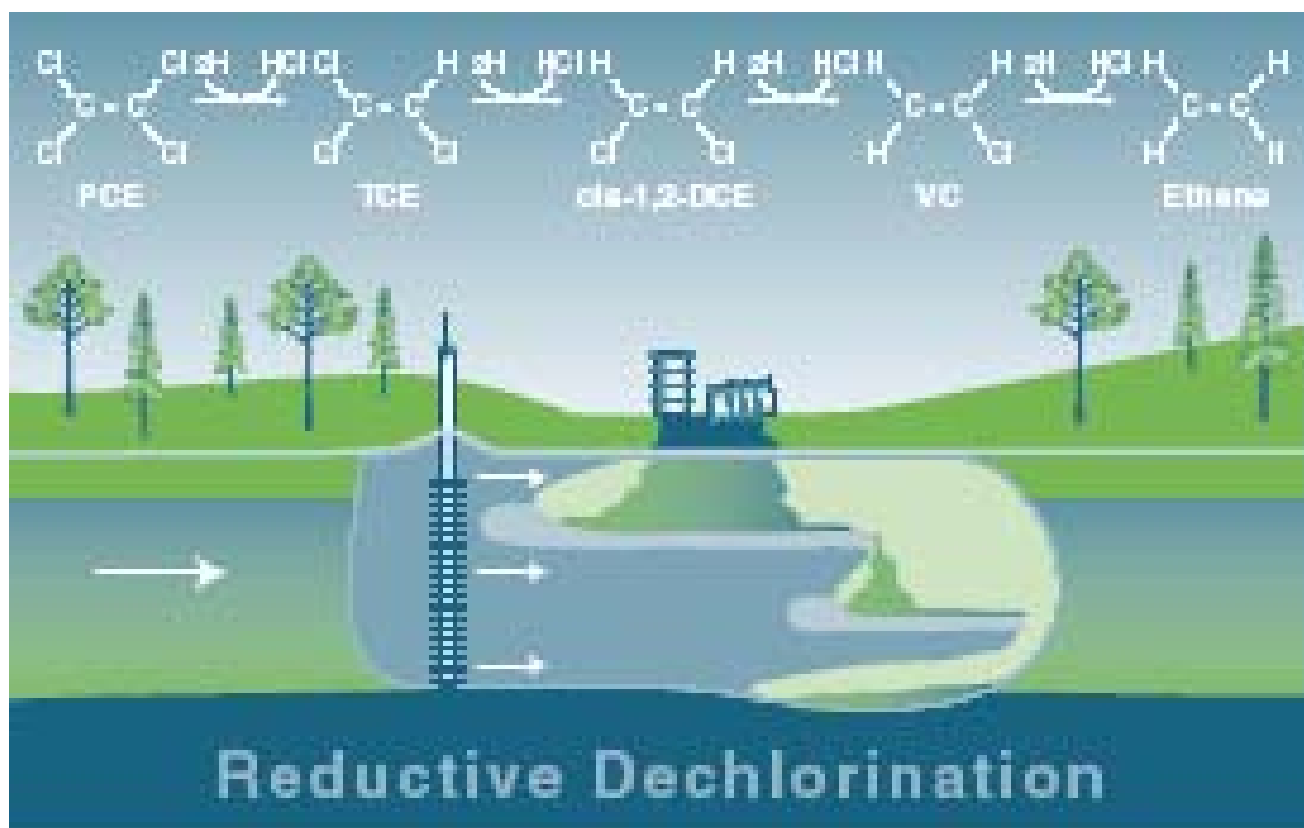


## Resource Guide

### In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones: A Resource Guide



May 2007

Prepared by  
The Interstate Technology & Regulatory Council  
Bioremediation of DNAPLs

## **ABOUT ITRC**

Established in 1995, the Interstate Technology & Regulatory Council (ITRC) is a state-led, national coalition of personnel from the environmental regulatory agencies of some 48 states and the District of Columbia, three federal agencies, tribes, and public and industry stakeholders. The organization is devoted to reducing barriers to, and speeding interstate deployment of better, more cost-effective, innovative environmental techniques. ITRC operates as a committee of the Environmental Research Institute of the States (ERIS), a Section 501(c)(3) public charity that supports the Environmental Council of the States (ECOS) through its educational and research activities aimed at improving the environment in the United States and providing a forum for state environmental policy makers. More information about ITRC and its available products and services can be found on the Internet at [www.itrcweb.org](http://www.itrcweb.org).

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# **Resource Guide**

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The Interstate Technology & Regulatory Council  
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# **IN SITU BIOREMEDIATION OF CHLORINATED ETHENE DNAPL SOURCE ZONES: A RESOURCE GUIDE**

## **1.0 INTRODUCTION**

### **1.1 Description of ITRC**

Established in 1995, the Interstate Technology & Regulatory Council (ITRC) is a state-led, national coalition of personnel from the environmental regulatory agencies of more than 40 states and the District of Columbia, three federal agencies, tribes, and public and industry stakeholders. The organization is devoted to reducing barriers to and speeding interstate deployment of, better, more cost-effective, innovative environmental techniques. ITRC operates as a committee of the Environmental Research Institute of the States (ERIS), a Section 501(c) (3) public charity that supports the Environmental Council of the States (ECOS) through its educational and research activities aimed at improving the environment in the United States and providing a forum for state environmental policy makers. Our network of more than 11,000 people from all aspects of the environmental community is a unique catalyst for dialogue between regulators and the regulated community to build and share technical knowledge about the selection, approval, and application of emerging technologies. Together, we're building the states' ability to expedite quality environmental decision making while protecting human health and the environment.

ITRC accomplishes its mission in two ways: it develops guidance documents and training courses to meet the needs of both regulators and environmental consultants, and it works with state representatives to ensure that ITRC products and services have maximum impact among state environmental agencies and technology users. The development of guidance documents and training courses is conducted through technical teams, led by state regulators and relies on broad-based participation from federal agencies, industry, academia, and other stakeholders in building collective knowledge and collaborative products. The Bioremediation of DNAPLs (BioDNAPL) Team is one of 15 ITRC technical teams. Its aim is to develop the technical and regulatory requirements for the bioremediation of DNAPLs, with an emphasis on DNAPLs associated with chlorinated ethenes. Chlorinated solvents were widely used throughout a number of industries leading to numerous environmental contamination problems, and both DoD and DOE face similar DNAPL contamination problems at many of their facilities. Current DNAPL remediation technologies require the use of energy (e.g. steam and heat), fluids (e.g. surfactants), or oxidants (e.g. potassium permanganate) to mobilize DNAPL for subsequent recovery and/or destruction. A potential advantage of bioremediation is that microorganisms – which can proliferate and attack the contaminant at or near the DNAPL interface without mobilization – might provide a far more efficient, effective and less costly remediation.

### 1.1.1 Training Courses

ITRC develops and delivers free, live, interactive, Internet-based training on emerging environmental technologies and approaches. We also partner with industry and other organizations to develop inexpensive classroom courses offered across the country. ITRC's cost-effective training has successfully reached more than 15,000 state, federal, industry, and other stakeholders. When asked about the impact of ITRC documents and training, 90% of respondents indicate that the knowledge they've gained will help them save time or money— usually both—and sometimes the savings amount to millions.

### 1.1.2 Consensus in the Environmental Community

Working in teams to create documents and training, ITRC participants leverage each other's expertise. The contentiousness that often characterizes relations between regulators and the regulated community dissipates as teams build understanding of the conditions under which new technologies should be applied, consensus about how they should be regulated, and confidence in their merits. Sharing problems, information, and lessons learned spreads news of successful solutions and increases deployments of the most appropriate technologies and approaches

### 1.1.3 Guidance Documents

ITRC's guidance documents include technology overviews, case studies, and technical/regulatory guidelines. These guidelines— often incorporating decision trees—suggest uniform data requirements for technology demonstrations or approvals. State concurrence with ITRC guidance makes the permitting process more uniform and efficient across states, helping technology consultants and vendors avoid the time and expense of meeting different requirements in each state where an innovative technology is proposed for use.

ITRC is bringing about a culture change in environmental decision making, replacing long-standing adversarial relationships with collaboration, consensus, and concurrence. State regulators are using ITRC guidance documents, training, and peer exchange to find creative ways to reduce regulatory barriers to new environmental technologies, cut approval time, and enhance their ability to make quality decisions. As a result, regulated industries and contractors are benefiting from reduced remediation costs and accelerated cleanup schedules. ITRC's ultimate beneficiary is the public—through a safer, healthier environment; redeveloped brownfields; and a better return on tax dollar.

More information about ITRC and its available products and services can be found on the Internet at [www.itrcweb.org](http://www.itrcweb.org).

## **1.2 Purpose of Resource Guide**

This resource guide provides a compilation of relevant scientific and technical literature on the bioremediation of chlorinated ethene Dense Non-Aqueous Phase Liquids (DNAPLs). In that regard, the resource guide is designed to help regulators, technology practitioners, site owners and others develop a consistent approach to the basic principles, terminology, and technical features of bioremediation. The guide attempts to

address the most critical aspects of the technology, but it is not intended to be an exhaustive treatise on the subject either in breadth or depth.

## 2.0 OVERVIEW OF BIOREMEDIATION OF DNAPL

In situ bioremediation, or ISB, is the use of microorganisms in the subsurface to degrade contaminants in place metabolically. In general, microbial metabolism requires a source of carbon, an electron donor, an electron acceptor, appropriate nutrients, and suitable environmental conditions.

This Resource Guide discusses in situ bioremediation of chlorinated ethene DNAPLs. We are aware that there are other types of DNAPLs (e.g., creosote) but this Reference Guide is focused mostly on the chlorinated ethenes because that is the group of contaminants on which most of the research has been conducted.

The potential for biodegradation of chlorinated organic solvents has been recognized since the early 1980's (Vogel et al. 1987). Anaerobic biodegradation in particular has been used commercially for natural and enhanced remediation of dissolved phase plumes (AFCEE 2004, USEPA 1998); however application of ISB to DNAPL source zones is still in the development phase. Based on a handful of field-scale demonstrations, ISB appears promising for cost-effectively shortening remediation timeframes for DNAPL source areas comprised primarily of residual or sorbed mass, but probably not for sites with significant drainable DNAPL mass. While the overall effectiveness of ISB for chlorinated ethene DNAPL source zone applications is still relatively poorly understood, emerging evidence from a limited number of laboratory and field studies completed to date indicate that ISB has the potential to address many DNAPL sites.

For chlorinated ethene DNAPLs, biodegradation is understood to occur through one or more of three different pathways, which may occur simultaneously in the subsurface:

1. the use of the contaminant as an electron acceptor, where the contaminant is reduced by the microbe but not used as a carbon source;
2. the use of the contaminant as an electron donor, where the contaminant is oxidized by the microbe, and the microbe obtains energy and organic carbon from the contaminant; and
3. by the process of co-metabolism, in which an enzyme or other factor used by the microbe for some other purpose fortuitously destroys the contaminant while providing no benefit to the microbe itself.

It is important to realize that bioremediation is not believed to work directly on the free-phase DNAPL. Instead, the technology relies on degradation and solubilization processes that occur near the water-DNAPL interface. Studies indicate that biomass can grow on the surface of some types of NAPL (Baldi et al, 1999), and adhesion of *Acinetobacter venatianus* to diesel fuel droplets are documented through studies using *in situ*

electrochemical and molecular probes (Applied Environmental Microbiology 65:2041-2048). The contaminant mass stored in the non-aqueous-phase must transfer into the aqueous phase before it can be subjected to the dechlorination processes. Enhanced bioremediation is the introduction of an electron donor and possibly non-indigenous microbes to enhance the removal of chloroethene DNAPLs through reductive dechlorination. Enhanced removal relies on inducing a steep concentration gradient between the DNAPL and the dissolved phases. In some cases, a separate phase donor (such as vegetable oil) may also be added to sequester the DNAPL in situ and foster biodegradation of DNAPL constituents as they solubilize over time.

Enhanced reductive dechlorination occurs through addition of an organic electron donor to facilitate the sequential transformation of chlorinated ethenes as follows: PCE → TCE → cis-DCE → VC → ethene. The partitioning coefficients ( $K_{oc}$ ) of degradation products decrease with each step in the transformation, thus each degradation product will be less sorptive than the previous degradation product. In addition, aqueous solubility increases from PCE to TCE to DCE. With these properties in mind, the mechanisms that are currently understood to enhance DNAPL mass removal during bioremediation of DNAPL source zones can be divided into three types (Sorenson, 2002), which are briefly described below.

1. Enhancement of the mass transfer rate during ISB resulting from an increase in concentration gradient due to contaminant degradation in the aqueous phase.
2. Enhanced mass transfer into the aqueous phase due to the changes in properties (primarily increased solubility) of the degradation products relative to the parent compounds.
3. Abiotic interaction of electron donor solutions with the contaminant mass (surfactants and co-solvent affects).

The complete dissolution of non-aqueous phase contaminant mass is limited by several factors, including the typically large amount of non-aqueous phase mass present, as compared to the aqueous phase, and the slow rate of dissolution. At some sites, significant destruction of contaminant mass in the source area can be achieved by increasing the rate of contaminant dissolution. However, even with dissolution rate increases, source areas at other sites are expected to persist for many decades, due to the large amount of non-aqueous phase mass present. Despite variation in source area characteristics, enhancing the contaminant dissolution rate remains a key process objective for bioremediation of source areas.

[Interstate Technology Regulatory Council \(2004\). Overview of In Situ Bioremediation of Chlorinated Ethene DNAPL Source Zones, Bioremediation of DNAPLs Team.](#)

This report was published by the Interstate Technology and Regulatory Council (ITRC). It is an overview document that includes discussions about technical considerations for In Situ Bioremediation (ISB) of chlorinated ethene DNAPLs, the current state of ISB technology application, the potential for ISB to achieve site remediation objectives, and the means to measure the progress and effectiveness of ISB of DNAPL contamination.

[Sutherson, S.S. and F.C. Payne, 2005. In Situ Remediation Engineering. CRC Press, Boca Raton, Florida.](#)

In Situ Remediation Engineering provides a comprehensive guide to the design and implementation of reactive zone methods for treatment of all major classes of groundwater contamination, including hydrophobic and miscible organics, metals, and other high-solubility inorganics. It teaches the fundamentals that underlie development of cost-effective reactive zone strategies, such as contaminant distribution and transport patterns, chemical and biological mechanisms that can be used to achieve oxidation, reduction, or precipitation of target compounds, and identification and management of by-products and secondary water quality impacts of treatment technologies. This book guides the selection of cost-effective remedial strategies and provides environmental engineers and scientists with tools to achieve optimal deployment of source area, reactive barrier, and site-wide treatments. It offers extensive coverage of remedial system operation, discussing reagent injection strategies, interpretation of process monitoring results for biological and chemical reactive zone systems, and impacts of treatment processes on aquifer hydraulic characteristics. The text emphasizes the innovative and experimental treatment technologies for emerging contaminants such as radionuclides, explosives, pharmaceutical compounds, perchlorate, chlorophenols, and solvent stabilizers.

[Sorenson, K.S., 2002. Enhanced Bioremediation for Treatment of Chlorinated Solvent Residual Source Areas, Chlorinated Solvent and DNAPL Remediation – Innovative Strategies for Subsurface Cleanup, ACS Symposium Series 837, American Chemical Society, Washington, D.C., pp. 119-131.](#)

Chlorinated Solvent and DNAPL Remediation addresses remediation of chlorinated solvents and dense nonaqueous phase liquids (DNAPLs) in groundwater and discusses remedial alternatives that are available for subsurface cleanup. Chlorinated Solvent and DNAPL Remediation: Innovative Strategies for Subsurface Cleanup focuses primarily on current technological developments and innovative applications for in situ remediation of chlorinated solvents including DNAPLs in soil and groundwater. However, this book also provides a general overview of all of the physical, chemical, and biological processes available for in situ remediation of groundwater contaminated with chlorinated solvents and DNAPLs. Chapters discuss surfactant flushing to enhance DNAPL removal; in situ chemical destruction by reduction processes involving zero valent iron or related

metals; in situ chemical destruction by advanced oxidation processes; and in situ biological destruction by enhanced anaerobic bioremediation or natural bioattenuation. This book also emphasizes zero valent iron-based strategies, including reaction geochemistry, permeable reactive barrier longevity, rejuvenation of iron walls, and emplacement technique. One chapter summarizes 10 years of permeable reactive barrier development and application. The controversial issues related to DNAPL remediation, including the concept that remediation of sites affected by DNAPL could be technically impractical, are reviewed. Another chapter focuses on the evolution of DNAPL remediation practice.

[Yang, Y. and P.L. McCarty, 2000. Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. Environmental Science and Technology, 34\(14\):2979-2984.](#)

One major problem with tetrachloroethene (PCE) contamination of aquifers is its ability to form dense, nonaqueous-phase liquids (DNAPL), which can act as a persistent contamination source for decades. Batch studies were performed to determine the potential for biological reductive PCE dehalogenation at high concentration and the effect on competing microorganisms, including methanogens and homoacetogens. Results show that PCE dehalogenation can be obtained at saturation concentration (>0.9 mM). Also, trichloroethene was dehalogenated up to 2.26 mM, and no apparent inhibitory effect on dehalogenation was found with *cis*-1,2-dichloroethene (cDCE) and ethene at the highest tested levels of 0.66 and 1.05 mM, respectively. However, such high concentrations of PCE, cDCE, and ethene were inhibitory to methanogens, and high concentrations of PCE were inhibitory to homoacetogens. Such inhibition is highly beneficial as it greatly diminished the competition by methanogens and homoacetogens for added electron donors, including hydrogen, resulting in highly efficient substrate utilization for dehalogenation. PCE DNAPL dehalogenation in a column study required less than 1 g of the electron donor pentanol to dehalogenate 1 g of PCE to cDCE (<2 mol of pentanol/mol of PCE). Additionally, DNAPL dissolution rate was significantly enhanced when directly coupled with biological dehalogenation.

[Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents, 2004. AFCEE](#)

Although enhanced in situ anaerobic bioremediation has been applied at hundreds of sites to date, it has yet to gain widespread regulatory acceptance as a proven technology primarily due to unfamiliarity with the technology and a lack of consistency in achieving remedial objectives. Therefore, the objectives of the Principles and Practices document are to describe the scientific basis of enhanced in situ anaerobic bioremediation and to summarize relevant site selection, design, and performance criteria for various engineered approaches to stimulate and enhance in situ biodegradation of chlorinated solvents. The document is not intended as a protocol to implement enhanced bioremediation. Rather, the document provides the information necessary to make

informed decisions as to when enhanced in situ anaerobic bioremediation is appropriate, and what specific enhanced bioremediation approaches may be suitable for achieving remedial goals.

[Moretti, L., 2005. In Situ Bioremediation of DNAPL Source Zones, report prepared for U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response Technology Innovation and Field Services Division](#)

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.

### 3.0 DNAPL CHARACTERIZATION

In order to make appropriate decisions about how to respond to the presence of contaminants at a site, the type, extent, and fate and transport of the site specific contaminants must be understood and characterized. Although the processes can be time consuming and complex, it is generally possible to accurately characterize dissolved phase organic contamination as the subsurface processes governing this contamination phase are relatively well understood and the necessary scientific tools needed to characterize the contaminants are readily available, generally understood and sophisticated.

At sites contaminated with dense non-aqueous phase liquids (DNAPLs), adequate site characterization can be extremely difficult. The physical and chemical processes governing DNAPL fate and transport in the subsurface are very complex and may cause DNAPLs to be distributed very differently than would be expected for dissolved phase contaminants. DNAPLs may be distributed independently of groundwater flow direction and saturation. DNAPL migration often follows tortuous pathways that may be caused by very minute heterogeneities in the subsurface geology that while very difficult to identify and characterize, have a significant effect on the DNAPL flow and distribution. DNAPLs may be found in large and small potentially mobile pools or, very commonly, as isolated and immobile ganglia. Subsurface investigations must be carefully planned. Proper techniques must be used to prevent detrimental impacts that can occur if investigation activities cause DNAPL migration to occur.

Characterizing DNAPL sites therefore requires tools and techniques that will allow the investigator to obtain enough information about the characteristics of the DNAPL, the subsurface conditions, the DNAPLs interaction with the subsurface geology, and the DNAPL fate and transport in the subsurface to make the necessary decisions whether or not and how to remediate the site. There are tools that exist to help with this process and more are being developed. The following references provide information on the physical and chemical processes governing the fate and transport of DNAPLs in the subsurface and an introduction to tools that can be used to help characterize DNAPL sites and allow site specific decision to be made and remediation to be planned.

[Crumbling, D., C, et. al. \(2001\). "Managing Uncertainty in Environmental Decisions: Applying the Concept of Effective Data at Contaminated Sites Could Reduce Costs and Improve Cleanups." Environmental Science and Technology, 35:404A–409A.](#)

This paper introduces the Triad Site Characterization concept of managing data uncertainty during characterization and discusses the concept that this process is much more than just reviewing the analytical methods. Data uncertainty includes understanding uncertainty caused by geology, media, sampling techniques, etc. Therefore, when considering data it is important to understand the uncertainty and address this. The paper also discusses the concept that with data uncertainty unavoidable, if there are a fixed amount of characterization resources available, it may better to analyze a large number of

samples by a potentially less analytically accurate method than a very few samples by a very accurate analytical method as long as the uncertainty in the analytical methods are understood. The results of a large number of analyses may give a better understanding of the site than just a few sample results would as long as uncertainty is known and understood and part of the site characterization planning.

[Cohen, R. M., and Mercer, J. W., \(1993\).DNAPL Site Evaluation. C.K. Smoley-CRC Press, Boca Raton, Fla.; EPA Publication EPA 600-R-93-022, 1993, from NTIS \(PB93-150217\)\].](#)

This Book provides a detailed and full discussion of DNAPLs. The abstract provided with the EPA cover for this book reads: "Dense nonaqueous-phase liquids (DNAPLs), especially chlorinated solvents, are among the most prevalent subsurface contaminants identified in ground-water supplies and at waste disposal sites." "The manual discusses the scope of the DNAPL problem, the properties of DNAPLs and subsurface media affecting DNAPL transport and fate, objectives and strategies for DNAPL site characterization, invasive and non-invasive methods of site characterization, and laboratory methods for characterizing fluid and media properties. The manual concludes with several case histories illustrating problems specific to DNAPL sites and priority research needs for improving DNAPL site characterization."

[Feenstra, S., D.M. Mackay, and J.A. Cherry. \(1991\). "A Method for Assessing Residual Napl Based on Organic Chemical Concentrations in Soil Samples." \*Ground Water Monitoring Review\*, 11\(2\)128–136.](#)

#### NAPL

This paper provides a simple quantitative tool that can be used to help assess whether or not chemical concentrations of a contaminant in soils indicate the presence of residual NAPL. The method uses general principles of chemical partitioning, site specific soil characteristics and contaminant concentrations to indicate if the contaminant concentrations are indicative of residual NAPL.

[Interstate Technology Regulatory Council, \(2003\). An Introduction to Characterizing Sites Contaminated With DNAPLs, Technical report prepared by the Interstate Technology and Regulatory Council's Dense Nonaqueous Phase Liquids Team](#)

This document provides an introduction to the concept of characterizing sites contaminated with DNAPLs. It is written for the non-technical party such as a concerned neighbor or attorney stakeholder and technical personnel just beginning their career. The document introduces the non technical party to the concepts of what DNAPL is, why it is hard to characterize, and techniques to characterize DNAPL sites. It also provides case studies and references to other more technical literature concerning this topic.

[Interstate Technology Regulatory Council, \(2003\). Technical and Regulatory Guidance for the Triad Approach: A New Paradigm for Environmental Project Management. Guidance document prepared by the Interstate Technology and Regulatory Council's Sampling, Characterization, and Monitoring Team](#)

This document introduces the Triad approach to conducting environmental work, which increases effectiveness and quality and reduces project costs. It details the three "legs" of the triad approach and lists its advantages and disadvantages. The Document also discusses Triad in context of regulatory issues surrounding its use and notes regulatory and organizational barriers to its use. Case studies that detail the use of the Triad methodology and concept are included in the appendix.

[Interstate Technology Regulatory Council, \(2006\) The Use of Direct-push Well Technology for Long-term Environmental Monitoring in Groundwater Investigations Guidance document prepared by the Interstate Technology and Regulatory Council's Sampling, Characterization, and Monitoring Team](#)

This document provides detailed information on the use of Direct Push well technology. While not designed directly for DNAPL investigation, this technology can be used very successfully as part of the investigation and management of a DNAPL bioremediation site. The following text taken from Executive Summary of the document provides a brief discussion: "This document provides technical and regulatory guidance concerning the use of Direct Push wells for long-term environmental groundwater monitoring. Direct Push wells offer the potential to save significant amounts of money and time for environmental groundwater monitoring. Equipment used to install such wells is usually smaller and lighter than conventional drilling rigs, so less damage is done to landowner's property. The quality of data from groundwater samples taken from Direct Push wells are comparable to those obtained from conventional wells. Despite these positive attributes, most states' regulations inadvertently prohibit their use for long-term groundwater monitoring and relegate their usage primarily to screening purposes."

[Kram, M. L., Keller, A. A., Rossabi, J., and Everett, L. G., \(2001\). "DNAPL Characterization Methods and Approaches, Part I: Performance Comparisons," \*Ground Water Monit. Remed.\* Vol. 21, No. 4, 109–23.](#)

This paper is the first part of a two part paper and "provides a comparison of the advantages and disadvantages of" DNAPL site characterization method currently in use to detect and characterize DNAPL contaminant source zones. "The objective is to determine which options are best to pursue based on site characteristics, method performance, and method costs. DNAPL characterization methods are grouped into approaches, which include site preparation, characterization, and data processing activities necessary to design an effective remediation system." The authors' comparison of the different approaches can help with the selection of characterization methods for a site and "assist with selection of appropriate site remediation management options"

[Kram, M. L., Keller, A. A., Rossabi, J., and Everett, L. G., \(2001\). "DNAPL Characterization Methods and Approaches, Part II: Cost Comparisons," \*Ground Water Monit. Remed.\* Vol. 22, No. 1: 46–61.](#)

This paper is the second part of a two part paper comparing DNAPL site characterization methods. It compares the costs of characterization approaches assessed in Part 1 using Unit Model Scenarios (UMSs) with "unit costs and assumptions related to labor,

equipment, and consumables" applied to technique specific costs for each approach for various UMSs.

[Kueper, B.H. and Wealthall, G.P. \(2003\). \*An Illustrated Handbook of DNAPL transport and fate in the subsurface\*. UK Environmental Agency R&D Report 133.](#)

From the executive summary: "The purpose of this handbook is to provide a user-friendly overview of the nature of DNAPL contamination in a UK context. It is intended to assist site investigators, site owners and regulators in conducting site investigations, conducting risk assessments and selecting remediation approaches. While this handbook reflects the state-of-the-art at the time of publication, it should be noted that the discipline of groundwater and soil contamination by hazardous organic liquids is evolving continuously and is relatively 'young' compared with many other areas of science and engineering. Readers are therefore advised to keep abreast of the new advances expected in the foreseeable future."

[Kueper, B. H., and McWhorter, D. B., \(1991\). "The Behavior of Dense, Nonaqueous Phase Liquids in Fractured Clay and Rock," \*Groundwater\* Vol. 29, No. 5.](#)

From the Abstract: "This paper examines the behavior of dense, nonaqueous phase liquids (DNAPLs) in fractured clay and rock". It discusses the conditions and mechanics of a DNAPL accumulating above, entering a water filled fracture, then migrating thorough the fracture. It includes numerical simulations to simulate and describe this process. It includes discussions of the conditions that control entry and flow including the properties of the DNAPL and the physical conditions of the aquifer.

[Pankow, J.F., and Cherry, J.A. \(eds\). \(1996\). \*Dense Chlorinated Solvents and other DNAPLs in Groundwater\*, Waterloo Press, Portland Oregon.](#)

This book contains 14 chapters, each of which are separate articles written by various authors. The chapters cover the concept of DNAPLS including background and history of the DNAPL problem, Conceptual Models of DNAPL behavior in the subsurface, mechanics and movement of DNAPLS in porous media, numerical simulation of movement of DNAPLS, experimental studies, vapor migration in the vadose zone, dissolution of DNAPLS in the subsurface, sorption of dissolved chlorinated solvents to aquifer media, chemical and microbiological degradation, effects of chlorinated solvents on clay permeability, physics of DNAPL migration in fractured media, effects of molecular diffusion on DNAPLS in fractured media, diagnosis and assessment of DNAPL sites, and concepts of DNAPL site remediation.

[Parker, B. L, Gillham, R. W., and Cherry, J. A., \(1994\). "Diffusive Disappearance of Immiscible-Phase Organic Liquids in Fractured Geologic Media," \*Groundwater\* Vol. 32, No.5, 805–20.](#)

This paper introduced the model of diffusion of NAPLs into the porous matrix between fractures in fractured rock aquifers contaminated by NAPLs. This paper revised the conceptual model of DNAPL flow, characterization, and remediation in fractured porous bedrock aquifers and helps explain the persistence of contamination in these aquifers.

[US Army Corps of Engineers \(1998\). Technical Project Planning \(TPP\) Process. Army Corps of Engineers publication: Engineer Manual 200-1-2, 31 August 1998.](#)

From the document: "This Engineer Manual (EM) describes the Technical Project Planning (TPP) process for identifying project objectives and designing data collection programs at hazardous, toxic, and radioactive waste sites, The TPP process helps ensure that the requisite type, quality, and quantity of data are obtained to satisfy project objectives that lead to informed decisions and site closeout."

[USEPA. \(2004\). Site Characterization Technologies for DNAPL Investigations, September 2004, EPA publication: EPA 542-R-04-017, September 2004](#)

This document introduces the DNAPL problem and discusses and provides references for DNAPL site characterization approaches and technologies including detailed discussions of the Triad approach to site characterization, using the technology "toolbox" to improve site characterization, and introductions and references to both non-geophysical and geophysical techniques used to for DNAPL site characterizations. It also presents brief case studies of the technologies being used on DNAPL sites, a brief discussion of the physical/geochemical behavior of DNAPLs, internet resources, and a list of technology vendors.

[USEPA. \(2003\).Using Dynamic Field Activities for On-Site Decision Making: A Guide for Project Managers EPA Publication: OSWER No. 9200.1-40, EPA/540/R-03/002 May 2003. US Govt. Print. Office, Washington D.C.](#)

US EPA OSWER guidance document for using Dynamic Field activities for on-site decision making. From the US EPA fact sheet for this document: "The primary purpose of this guidance is to provide contaminated site project managers with an overview of the information they need to oversee the effective implementation of dynamic field activities at their sites. Additionally, the guidance should help educate other key decision-makers (e.g., relevant U.S. EPA personnel, contractors, other federal and state agencies, and potentially responsible parties) about their roles in implementing this process."

Water Science and Technology Board, 2004, [Contaminants in the Subsurface: Source Zone Assessment and Remediation](#) The National Academies Press, Washington, D.C.

This paper provides a detailed assessment of issues involving contaminant source zone characterization and remediation. It focuses primarily on organic contaminants and provides a definition of contaminant source zone that is appropriate for DNAPL source zones.

## 4.0 DNAPL DISSOLUTION AND PARTITIONING PHENOMENA

Key to an understanding of the impacts of bioremediation on removal of mass from source areas are the phenomena of dissolution (i.e., mass transfer from the non-aqueous to the aqueous phase) and contaminant partitioning. Dissolution is a dynamic process that depends on many factors – e.g. both the geometry and architecture of DNAPL in the subsurface and the local rate of groundwater flow through the porous medium containing DNAPL. A better understanding of partitioning – or the concentration between phases at equilibrium – and its importance during enhanced bioremediation, is beginning to emerge.

### 4.1 Theoretical Background

[Powers, S.E., L.M. Abriola, and W.J. Weber, Jr., 1994. An experimental investigation of nonaqueous phase liquid dissolution in saturated subsurface systems: Transient mass transfer rates, \*Water Resources Research\*, 30\(2\):321-332.](#)

One-dimensional dissolution experiments were used to determine an empirical mass transfer rate coefficient that is strongly correlated with groundwater velocity, porous medium properties (grain size and grain size distribution) and volumetric NAPL saturation. Predicted times for NAPL removal and reduction in groundwater concentrations are significantly longer than predicted by equilibrium dissolution. Using this mass transfer model predicts aqueous concentrations that agree closely with the experimental data.

[Frind, E.O., J.W. Molson, M. Schirmer, and N. Guiguer, 1999. Dissolution and mass transfer of multiple organics under field conditions: the Borden emplaced source, \*Water Resources Research\*, 35\(3\):683-694.](#)

A three dimensional flow and transport model incorporating rate-limited dissolution from a multi-component residual DNAPL source was used to simulate a 1000-day field experiment in which a small residual DNAPL source zone was installed in a shallow water-table aquifer. The simulations indicate that mass transfer is essentially an equilibrium process within the source zone and other processes (e.g., low source permeability and the declining solubility of DNAPL constituents) explain the low concentrations observed immediately downgradient of the source zone. The estimated times for complete source removal through dissolution are 25-35 years

### 4.2 DNAPL Solubility and Partitioning Effects

[Broholm, K. and S. Feenstra, Laboratory measurements of the aqueous solubility of mixtures of chlorinated solvents, \*Environmental Toxicology and Chemistry\*, 14\(1\):9-15, 1995.](#)

The theoretical basic for calculating the effective solubility of chlorinated solvents in a multi-component NAPL is provided. Experimental observations of solubility in binary

and ternary solvent mixtures agreed closely with calculated effective solubilities, except at relatively low mole fractions, indicating that the assumption of ideal partitioning behavior is valid.

[Carr, C.S., S.Garg, and J.B. Hughes, 2000. Effect of dechlorinating bacteria on the longevity and composition of PCE-containing non-aqueous phase liquids under equilibrium dissolution conditions, Environmental Science and Technology, 34\(6\):1088-1094.](#)

Enhanced dissolution by biodegradation was investigated in continuous-flow stirred-tank reactors (CFSTRs) bioaugmented with a PCE dechlorinating culture and a model NAPL (PCE in tridecane). Following amendment with formate (10 mM), PCE was dechlorinated to trichloroethene and cis-dichloroethene. Partitioning of chlorinated products from the aqueous phase into the NAPL changed the NAPL composition and was identified as a mechanism potentially prolonging the longevity of the DNAPL during remediation. The importance of this effect depends on the dechlorination end product (TCE partitions in PCE NAPL more strongly than cis-DCE, VC or ethene).

## 5.0 MICROBIOLOGY OF BIOREMEDIATION

The general mechanisms for chlorinated ethene biodegradation have been recognized since the early 1980's. These include aerobic cometabolism, aerobic oxidation of less chlorinated compounds (particularly VC), and anaerobic reductive dechlorination. The review prepared by Vogel and McCarty (1987) summarized what was known of these mechanisms at the time. This review remains the best general introduction, although there has been a considerable amount of increased knowledge regarding the physiology and identity of the microorganisms involved in the years since.

Cometabolism (or co-oxidation) of chlorinated ethenes by aerobic bacteria, via monooxygenase enzymes, can completely degrade TCE (e.g., [Fathepure et al., 1987](#); [Little et al., 1988](#); [Nelson et al., 1986](#)), and apparently also PCE (Ryoo DH, Shim H, Canada K, Barbieri P, Wood TK (2000). Aerobic degradation of tetrachloroethylene by toluene-o-xylene monooxygenase of *Pseudomonas stutzeri* OX1. Nat. Biotechnol. 18: 775-778.). Despite repeated efforts, using this process for commercial remediation has proven difficult.

Aerobic oxidation of DCE and VC has been suspected for many years, but organisms capable of growth on VC have only recently been identified (Mattes et al., 2004). This process may be occurring naturally at the aerobic fringes of plumes, and it may be enhanced in some cases to reduce plume lengths. Anaerobic oxidation of VC may also occur in redox transition zones where bioavailable Fe III is present. But it is not likely that these processes will be useful for treating source zones, given the demands for oxygen and the presence of parent compounds in most source zones.

With respect to bioremediation of DNAPL sources, only the reductive dechlorination pathway has been found to be significant to date. This review therefore focuses on that pathway, and primarily summarizes the work performed over approximately the last 10 years that has laid the groundwork for enhanced bioremediation of DNAPL source zones.

### 5.1 Factors influencing the rate and extent of reductive dechlorination

[Smatlak, C.R., J.M. Gossett, and S.H. Zinder, 1996. Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture, Environmental Science and Technology, 30:2850-2858.](#)

Anaerobic microorganisms that reductively dechlorinate tetrachloroethene (PCE) must often compete with methanogens for H<sub>2</sub>. This study compared the kinetics of H<sub>2</sub> utilization between the two types of organisms at 35 °C under conditions of continuous agitation. Limiting levels of H<sub>2</sub> were administered to 160-mL serum bottles seeded with a PCE/butyric acid enrichment culture; H<sub>2</sub>, methane, and vinyl chloride were tracked over time using headspace samples. Measured half-velocity constants with respect to H<sub>2</sub>-  $K_s$

(H<sub>2</sub>) values ± 95% CI-for methanogenesis and dechlorination were 960 ± 180 and 100 ± 50 nM, respectively. Mass-transfer equations were used to calculate aqueous H<sub>2</sub> concentrations at the half-velocity point from headspace measurements. The possible effect on K<sub>s</sub> (H<sub>2</sub>) values arising from interconversion between H<sub>2</sub> and formate through an active formate/H<sub>2</sub> lyase system was examined by comparing results from formate-fed and H<sub>2</sub>-fed bottles. Only methanogens in the culture were apparently capable of using formate; hence, the measured methanogenic K<sub>s</sub>(H<sub>2</sub>) was dependent on which electron donor was administered. The nearly 10-fold difference in K<sub>s</sub>(H<sub>2</sub>) between methanogens and dechlorinators suggests that the deliberate choice of an electron donor whose fermentation results in a slow, steady, and low-level release of H<sub>2</sub> over time could maximize dechlorination potential while minimizing methanogenic competition for H<sub>2</sub>.

[Duhamel, M., S.D. Wehr, L. Yu, H. Rizvi, S. Seepersad, S. Dworatzek, E.E. Cox, and E.A. Edwards, 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene, and vinyl chloride, Water Research, 36:4193-4202.](#)

An anaerobic mixed microbial culture was enriched from soil and groundwater taken from a site contaminated with trichloroethene (TCE). This enrichment culture was divided into four subcultures amended separately with either perchloroethene (PCE), TCE, cis-dichloroethene (cDCE) or vinyl chloride (VC). In each of the four subcultures, the chlorinated ethenes were rapidly, consistently, and completely converted to ethene at rates of 30-50 micromol/l of culture per day, or an average 160 micro-electron equivalents/l of culture per day. These cultures were capable of sustained and rapid dechlorination of VC, and could not dechlorinate 1,2-dichloroethane, differentiating them from *Dehalococcoides ethenogenes*, the only known isolate capable of complete dechlorination of PCE to ethene. Chloroform (CF) and 1,1,1-trichloroethane, frequent groundwater co-contaminants with TCE and PCE, inhibited chlorinated ethene dechlorination. Most strongly inhibited was the final conversion of VC to ethene, with complete inhibition occurring at an aqueous CF concentration of 2.5 microM. Differences in rates and community composition developed between the different subcultures, including the loss of the VC enrichment culture's ability to dechlorinate PCE. Denaturing gradient gel electrophoresis of amplified bacterial 16S rRNA gene fragments identified three different DNA sequences in the enrichment cultures, all phylogenetically related to *D. ethenogenes*. Based on the PCR-DGGE results and substrate utilization patterns, it is apparent that significant mechanistic differences exist between each step of dechlorination from TCE to ethene, especially for the last important dechlorination step from VC to ethene.

[Freedman, D.L. and J.M. Gossett, 1989. Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions. Applied and Environmental Microbiology, 55\(9\): 2144-2151.](#)

A biological process for remediation of groundwater contaminated with tetrachloroethylene (PCE) and trichloroethylene (TCE) can only be applied if the transformation products are environmentally acceptable. Studies with enrichment cultures of PCE- and TCE-degrading microorganisms provide evidence that, under methanogenic conditions, mixed cultures are able to completely dechlorinate PCE and TCE to ethylene,

a product which is environmentally acceptable. Radiotracer studies with [14C]PCE indicated that [14C]ethylene was the terminal product; significant conversion to 14CO<sub>2</sub> or 14CH<sub>4</sub> was not observed. The rate-limiting step in the pathway appeared to be conversion of vinyl chloride to ethylene. To sustain reductive dechlorination of PCE and TCE, it was necessary to supply an electron donor; methanol was the most effective, although hydrogen, formate, acetate, and glucose also served. Studies with the inhibitor 2-bromoethanesulfonate suggested that methanogens played a key role in the observed biotransformations of PCE and TCE.

[He, J., K.M. Ritalahti, M.R. Aiello, and F.E. Löffler, 2003. Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a Dhc Species, \*Applied and Environmental Microbiology\*, 69:996-1003.](#)

A major obstacle in the implementation of the reductive dechlorination process at chloroethene-contaminated sites is the accumulation of the intermediate vinyl chloride (VC), a proven human carcinogen. To shed light on the microbiology involved in the final critical dechlorination step, a sediment-free, nonmethanogenic, VC-dechlorinating enrichment culture was derived from tetrachloroethene (PCE)-to-ethene-dechlorinating microcosms established with material from the chloroethene-contaminated Bachman Road site aquifer in Oscoda, Mich. After 40 consecutive transfers in defined, reduced mineral salts medium amended with VC, the culture lost the ability to use PCE and trichloroethene (TCE) as metabolic electron acceptors. PCE and TCE dechlorination occurred in the presence of VC, presumably in a cometabolic process. Enrichment cultures supplied with lactate or pyruvate as electron donor dechlorinated VC to ethene at rates up to 54  $\mu\text{mol liter}^{-1}\text{day}^{-1}$ , and dichloroethenes (DCEs) were dechlorinated at about 50% of this rate. The half-saturation constant ( $K_S$ ) for VC was 5.8  $\mu\text{M}$ , which was about one-third lower than the concentrations determined for *cis*-DCE and *trans*-DCE. Similar VC dechlorination rates were observed at temperatures between 22 and 30°C, and negligible dechlorination occurred at 4 and 35°C. Reductive dechlorination in medium amended with ampicillin was strictly dependent on H<sub>2</sub> as electron donor. VC-dechlorinating cultures consumed H<sub>2</sub> to threshold concentrations of 0.12 ppm by volume. 16S rRNA gene-based tools identified a *Dehalococcoides* population, and *Dehalococcoides*-targeted quantitative real-time PCR confirmed VC-dependent growth of this population. These findings demonstrate that *Dehalococcoides* populations exist that use DCEs and VC but not PCE or TCE as metabolic electron acceptors.

[Maymó-Gatell, X., T. Anguish, and S. H. Zinder, 1999. Reductive Dechlorination of Chlorinated Ethenes and 1,2-Dichloroethane by "\*Dehalococcoides ethenogenes\*" 195, \*Applied and Environmental Microbiology\*, 65:3108-3113](#)

and

[Maymo-Gatell, X., I. Nijenhuis, and S.H. Zinder. 2001. Reductive dechlorination of \*cis\*-1,2-dichloroethene and vinyl chloride by "\*Dehalococcoides ethenogenes\*." \*Environmental Science & Technology\*, 35:516–521.](#)

Studied the first known dechlorinating bacterium, *Dehalococcoides ethenogenes* strain 195, and showed that it obtains energy from all chloroethene dechlorination steps (PCE to TCE, TCE to DCE, DCE to VC) with the exception of the final step from VC to ethene, which occurs cometabolically, resulting in the accumulation of VC and slower conversion of VC to ethene.

[Hendrickson, E. R., J.A. Payne, R. M. Young, M. G. Starr, M. P. Perry, S. Fahnestock, D. E. Ellis, and R. C. Ebersole, 2002. Molecular Analysis of \*Dehalococcoides\* 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe. \*Applied and Environmental Microbiology\*, 68:485-495](#)

Studied the distribution of *Dehalococcoides* at numerous sites in the U.S. and elsewhere, using 16S rRNA analysis. Noted that it was not found everywhere. Also it found that if the *Dehalococcoides* was not present at a site, then dechlorination past cis-DCE and VC to ethene did not occur.

[Krajmalnik-Brown R., T. Hölscher, I. N. Thomson, F. M. Saunders,<sup>1</sup> K. M. Ritalahti,<sup>1</sup> and F. E. Löffler, 2004. Genetic Identification of a Putative Vinyl Chloride Reductase in \*Dehalococcoides\* sp. Strain BAV1, \*Applied and Environmental Microbiology\*, 70:6347-6351.](#)

Studied vinyl chloride reduction by *Dehalococcoides ethenogenes*, strain BAV-1. Mapped the vinyl chloride reductase gene and developed a probe for the *vcr* gene. Showed that the *vcr* gene was different than the one found by Muller et al., and suggested there may in fact be numerous *vcr* genes.

[Sun, B., B. N. Griffin, H. L. Ayala-del-Rio, S. A. Hashsham, and J. M. Tiedje, 2002. Microbial dehalorespiration with 1,1,1-trichloroethane. \*Science\* 298:1023–1025.](#)

Isolated an organism capable of reductive dechlorination of 1,1,1-TCA. Found that the dechlorination was coupled to growth. The bacterium was identified as a *Dehalobacter* sp., and was named strain TCA1.

[Yang, Y. R. and P. L. McCarty, 1998. Competition For Hydrogen Within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. \*Environmental Science & Technology\*, 32:3591-3597.](#)

Studied competition between methanogens and dechlorinators. Found that *Dehalococcoides* competitively utilize hydrogen at concentrations below those supporting methanogenesis, and suggested that hydrogen concentrations should be controlled to favor dechlorination.

## **5.2 Concentration Tolerance and inhibitory contaminants**

[Adamson, D.T., J.M. McDade, and J.B. Hughes, 2003. Inoculation of a DNAPL source zone to initiate reductive dechlorination of PCE, \*Environmental Science and Technology\*, 37\(11\): 2525-2533.](#)

The ability to inoculate a PCE-NAPL source zone with no prior dechlorinating activity was examined using a near field-scale simulated aquifer. A known mass of PCE was added to establish a source zone, and the groundwater was depleted of oxygen using acetate and lactate prior to culture addition. An active and stable dechlorinating culture was used as an inoculum, and dechlorination activity was observed within 2 weeks following culture transfer. PCE reduction to TCE and *cis*-DCE was observed initially, and the formation of these compounds was accelerated by the addition of a long-term source of hydrogen (Hydrogen Releasing Compound). *cis*-DCE was the predominant chlorinated ethene present in the effluent after 225 days of operation, and production of VC and ethene lagged the formation of TCE and *cis*-DCE. However, dechlorination extent continued to improve over time, and VC eventually became a major product, suggesting that reinoculation was unnecessary. The detection of *Dehalococcoides* species in the source culture and in the simulated aquifer postinoculation indicated that the metabolic capability to dechlorinate beyond *cis*-DCE ( $t = 86$  days and  $t = 245$  days) was present. Elevated levels of TCE and *cis*-DCE were present in the source zone, but neither VC nor ethene was detected in the vicinity of NAPL. The results of this research indicated that adding dechlorinating cultures may be useful in the application of source zone bioremediation but that dechlorination beyond *cis*-DCE may be limited to regions downgradient of the source zone. (232 mg/L PCE to ethane.)

[Duhamel, M., S.D. Wehr, L. Yu, H. Rizvi, S. Seepersad, S. Dworatzek, E.E. Cox, and E.A. Edwards, 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene, and vinyl chloride, Water Research, 36:4193-4202.](#)

An anaerobic mixed microbial culture was enriched from soil and groundwater taken from a site contaminated with trichloroethene (TCE). This enrichment culture was divided into four subcultures amended separately with either perchloroethene (PCE), TCE, *cis*-dichloroethene (cDCE) or vinyl chloride (VC). In each of the four subcultures, the chlorinated ethenes were rapidly, consistently, and completely converted to ethene at rates of 30-50 micromol/l of culture per day, or an average 160 micro-electron equivalents/l of culture per day. These cultures were capable of sustained and rapid dechlorination of VC, and could not dechlorinate 1, 2-dichloroethane, differentiating them from *Dehalococcoides* ethenogenes, the only known isolate capable of complete dechlorination of PCE to ethene. Chloroform (CF) and 1,1,1-trichloroethane, frequent groundwater co-contaminants with TCE and PCE, inhibited chlorinated ethene dechlorination. Most strongly inhibited was the final conversion of VC to ethene, with complete inhibition occurring at an aqueous CF concentration of 2.5 microM. Differences in rates and community composition developed between the different subcultures, including the loss of the VC enrichment culture's ability to dechlorinate PCE. Denaturing gradient gel electrophoresis of amplified bacterial 16S rRNA gene fragments identified three different DNA sequences in the enrichment cultures, all phylogenetically related to *D. ethenogenes*. Based on the PCR-DGGE results and substrate utilization patterns, it is apparent that significant mechanistic differences exist between each step of dechlorination from TCE to ethene, especially for the last important dechlorination step from VC to ethene. (133 mg/L PCE to ethene, 197 mg/L TCE to ethene, dechlorinating

inhibited by 300 ug/L chloroform. Studied a mixed consortium identified as KB-1. Found that the culture could tolerate chlorinated ethene concentrations typically found at a DNAPL source zone. Specifically, found that the culture promoted dechlorination of PCE, TCE, cis-DCE and VC at initial concentrations of 132, 197, 77, and 87 mg/L, respectively, in microcosm studies.)

[Isalou, M., B.E. Sleep, and S.N. Liss, 1998. Biodegradation of high-concentrations of tetrachloroethene in a continuous-flow column system. Environmental Science and Technology, 32:3579-3585.](#)

A long-term (2.5 years) study of the anaerobic biodegradation of high concentrations of perchloroethylene (PCE) was carried out in a continuously operated laboratory column filled with sand which was inoculated with biomass from an anaerobic digester. Concentrations of PCE fed to the column were increased from 12  $\mu\text{M}$  to over 600  $\mu\text{M}$  over 21 months, with methanol added as electron donor. Vinyl chloride (VC) was the terminal product of PCE dechlorination for the first 21 months at which point significant conversion of VC to ethylene (ETH) was detected. The onset of ETH production coincided with acetogenesis becoming the primary pathway for methanol metabolism. ETH production occurred in the column in the presence of PCE and TCE. Varying methanol:PCE molar ratios from 1.4 to 7.5 had little effect on the transformation of PCE and TCE to VC. The degradation of VC to ETH was much more sensitive, and VC accumulated when the methanol:PCE molar ratio dropped below 5.0. Withdrawal of PCE from the system for a 5 month period and maintenance of the column on methanol alone did not result in the loss of PCE degradation capability of the consortium. (100 mg/L PCE to ethane; reported activity in saturated columns inoculated with TM-1, a dechlorinating culture derived from anaerobic digester sludge. PCE dechlorination was sustained at influent concentrations as high as 99 mg/L.)

[Maymo-Gatell, X., Y.T. Chien, J.M. Gossett, and S.H. Zinder, 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene, Science, 276: 1568–1571.](#)

Tetrachloroethene is a prominent groundwater pollutant that can be reductively dechlorinated by mixed anaerobic microbial populations to the nontoxic product ethene. Strain 195, a coccoid bacterium that dechlorinates tetrachloroethene to ethene, was isolated and characterized. Growth of strain 195 with  $\text{H}_2$  and tetrachloroethene as the electron donor and acceptor pair required extracts from mixed microbial cultures. Growth of strain 195 was resistant to ampicillin and vancomycin; its cell wall did not react with a peptidoglycan-specific lectin and its ultrastructure resembled S-layers of Archaea. Analysis of the 16S ribosomal DNA sequence of strain 195 indicated that it is a eubacterium without close affiliation to any known groups. (Dechlorination inhibited by 200 ug/L chloroform or 3 mg/L 1,1,1-TCA Studied the factors affecting dechlorination activity by strain 195. Found that the final step, VC to ethene, occurred slowly in a cometabolic process. Demonstrated the ability to dechlorinate at relatively high PCE concentrations, but also showed that other VOCs, notably chloroform and 1,1,1-TCA, caused complete inhibition, at concentrations of 450  $\mu\text{g/L}$  (3.8  $\mu\text{M}$ ) and 700  $\mu\text{g/L}$  (5.2  $\mu\text{M}$ ), respectively.)

[Nielsen, R.B. and J.D. Keasling, 1999. Reductive dechlorination of chlorinated ethene DNAPLs by a culture enriched from contaminated groundwater, \*Biotechnology and Bioengineering\* 62:160-165.](#)

A microbial culture enriched from a trichloroethene-contaminated groundwater aquifer reductively dechlorinated trichloroethene (TCE) and tetrachloroethene (PCE) to ethene. Initial PCE dechlorination rate studies indicated a first-order dependence with respect to substrate at low PCE concentrations, and a zero-order dependence at high concentrations. Studies of TCE and vinyl chloride (VC) dechlorination indicated a first-order dependence at all substrate concentrations. VC had little or no effect on the initial rate of TCE dechlorination. With subsaturating concentrations of chlorinated ethenes, nearly stoichiometric amounts of the toxic intermediate vinyl chloride accumulated prior to its dechlorination to ethene. In contrast, under saturating conditions, in which a dense, nonaqueous-phase liquid existed in equilibrium with the aqueous phase, the chlorinated ethene was dechlorinated to ethene, at a rapid rate, with the accumulation of relatively small amounts of chlorinated intermediates. © 1999 John Wiley & Sons, Inc. *Biotechnol Bioeng* 62: 160-165, 1999. (Both PCE and TCE degraded to ethene in the presence of DNAPL (i.e., PCE 232 mg/L, TCE 1105 mg/L))

[Yang, Y., and P.L. McCarty, 2000. Biologically enhanced dissolution of tetrachloroethene DNAPL, \*Environmental Science and Technology\*, 34\(14\)2979-2984.](#)

One major problem with tetrachloroethene (PCE) contamination of aquifers is its ability to form dense, nonaqueous-phase liquids (DNAPL), which can act as a persistent contamination source for decades. Batch studies were performed to determine the potential for biological reductive PCE dehalogenation at high concentration and the effect on competing microorganisms, including methanogens and homoacetogens. Results show that PCE dehalogenation can be obtained at saturation concentration (>0.9 mM). Also, trichloroethene was dehalogenated up to 2.26 mM, and no apparent inhibitory effect on dehalogenation was found with *cis*-1,2-dichloroethene (cDCE) and ethene at the highest tested levels of 0.66 and 1.05 mM, respectively. However, such high concentrations of PCE, cDCE, and ethene were inhibitory to methanogens, and high concentrations of PCE were inhibitory to homoacetogens. Such inhibition is highly beneficial as it greatly diminished the competition by methanogens and homoacetogens for added electron donors, including hydrogen, resulting in highly efficient substrate utilization for dehalogenation. PCE DNAPL dehalogenation in a column study required less than 1 g of the electron donor pentanol to dehalogenate 1 g of PCE to cDCE (<2 mol of pentanol/mol of PCE). Additionally, DNAPL dissolution rate was significantly enhanced when directly coupled with biological dehalogenation. (149 mg/L PCE, 297 mg/L TCE dechlorinated; 4 mg/L ethene sufficient to inhibit methanogenesis).

### 5.3 Bioaugmentation & the role of dehalococcoides

[Cupples, A.M., A.M Spormann, and P.L. McCarty, 2003. Growth of a Dehalococcoides-like microorganism on vinyl chloride and cis-dichloroethene as electron acceptors as determined by competitive PCR. Applied and Environmental Microbiology, 69:953-959.](#)

A competitive PCR (cPCR) assay targeting 16S ribosomal DNA was developed to enumerate growth of a *Dehalococcoides*-like microorganism, bacterium VS, from a mixed culture catalyzing the reductive dehalogenation of *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC), with hydrogen being used as an electron donor. The growth of bacterium VS was found to be coupled to the dehalogenation of VC and cDCE, suggesting unique metabolic capabilities. The average growth yield was  $(5.2 \pm 1.5) \times 10^8$  copies of the 16S rRNA gene/ $\mu\text{mol}$  of  $\text{Cl}^-$  (number of samples, 10), with VC being used as the electron acceptor and hydrogen as the electron donor. The maximum VC utilization rate ( $\hat{q}$ ) was determined to be  $7.8 \times 10^{-10}$   $\mu\text{mol}$  of  $\text{Cl}^-$  ( $\text{copy}^{-1} \text{ day}^{-1}$ ), indicating a maximum growth rate of  $0.4 \text{ day}^{-1}$ . These average growth yield and  $\hat{q}$  values agree well with values found previously for dechlorinating cultures. Decay coefficients were determined with growth ( $0.05 \text{ day}^{-1}$ ) and no-growth ( $0.09 \text{ day}^{-1}$ ) conditions. An important limitation of this cPCR assay was its inability to discriminate between active and inactive cells. This is an essential consideration for kinetic studies.

[Duhamel, M., S.D. Wehr, L. Yu, H. Rizvi, S. Seepersad, S. Dworatzek, E.E. Cox, and E.A. Edwards, 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene, and vinyl chloride. Water Research, 36:4193-4202.](#)

An anaerobic mixed microbial culture was enriched from soil and groundwater taken from a site contaminated with trichloroethene (TCE). This enrichment culture was divided into four subcultures amended separately with either perchloroethene (PCE), TCE, *cis*-dichloroethene (cDCE) or vinyl chloride (VC). In each of the four subcultures, the chlorinated ethenes were rapidly, consistently, and completely converted to ethene at rates of 30-50  $\mu\text{mol/l}$  of culture per day, or an average 160 micro-electron equivalents/ $\text{l}$  of culture per day. These cultures were capable of sustained and rapid dechlorination of VC, and could not dechlorinate 1,2-dichloroethane, differentiating them from *Dehalococcoides* ethenogenes, the only known isolate capable of complete dechlorination of PCE to ethene. Chloroform (CF) and 1,1,1-trichloroethane, frequent groundwater co-contaminants with TCE and PCE, inhibited chlorinated ethene dechlorination. Most strongly inhibited was the final conversion of VC to ethene, with complete inhibition occurring at an aqueous CF concentration of 2.5  $\mu\text{M}$ . Differences in rates and community composition developed between the different subcultures, including the loss of the VC enrichment culture's ability to dechlorinate PCE. Denaturing gradient gel electrophoresis of amplified bacterial 16S rRNA gene fragments identified three different DNA sequences in the enrichment cultures, all phylogenetically related to *D. ethenogenes*. Based on the PCR-DGGE results and substrate utilization patterns, it is apparent that significant mechanistic differences exist between each step of dechlorination from TCE to ethene, especially for the last important dechlorination step from VC to ethene.

[Ellis, D. E., E. J. Lutz, J. M. Odom, R. L. Buchanan, Jr., C. L. Bartlett, M. D. Lee, M. R. Harkness, and K. A. Deweerd, 2000. Bioaugmentation for accelerated in situ anaerobic bioremediation. Environmental Science and Technology, 34:2254-2260.](#)

The first peer-reviewed demonstration of bioaugmentation to treat chlorinated solvents under field conditions (the controlled test cells at Dover AFB, DE). The pilot test was preceded by lab microcosm and column studies (Harkness et al., 1999) that showed cis-DCE stall unless a mixed culture containing *Dehalococcoides ethenogenes* was added. The pilot treatment area was fed lactate for 269 days, during which time TCE was stoichiometrically dechlorinated to cis-DCE. VC and ethene were not produced during this interval. DCE was completely reduced to ethene only after the aquifer was amended with *Dehalococcoides* (the Pinellas strain used in the earlier tests).

[ESTCP, 2006.](#)

A recent summary of the development and current status of bioaugmentation for chlorinated ethene remediation. It includes a discussion of the scientific basis for bioaugmentation, as well as some guidance for selecting and implementing this technology at specific sites. There is a brief discussion of the “emerging” practice of bioaugmentation for DNAPL source treatment.

[Lendvay, J.M., F. E. Löffler, M. Dollhopf, M. R. Aiello, G. Daniels, B. Z. Fathepure, M. Gebhard, R. Heine, R. Helton, J. Shi, R. Krajmalnik-Brown, C. L. Major, Jr., M. J. Barcelona, E. Petrovskis, R. Hickey, J. M. Tiedje, and P. Adriaens. Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation. Environmental Science and Technology, 37:1422-1431](#)

A carefully-controlled field test of the effects of bioaugmentation at the Bachman Road site in Michigan. Two test plots (4.6 x 5.5 m) were constructed perpendicular to groundwater flow, separated by one plot of the same size. On day 29, 200 L (108 cell/mL) of the Bachman Road Culture was introduced into the bioaugmentation plot. Bioaugmentation resulted in a significant reduction in the time needed to achieve complete dechlorination to ethene as compared to the biostimulation-only plot. Complete dechlorination of PCE to ethene was achieved within 6 weeks after inoculation in the bioaugmentation plot. In the test plot, it took much longer for ethene to be observed at all, and at the end of testing (after 4 months of operation), approximately 75% of the PCE was converted to ethene in the biostimulation plot. Dechlorination in the bioaugmentation plot was demonstratively linked to the presence of *Dehalococcoides*.

[Major, D. W., M. L. McMaster, E. E. Cox, E. A. Edwards, S. M. Dworatzek, E. R. Hendrickson, M. G. Starr, J. A. Payne, and L. W. Buonamici, 2002. Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. Environmental Science and Technology 36:5106–5116.](#)

A successful field demonstration of bioaugmentation for treating dissolved-phase PCE, TCE and cis-DCE at Kelly AFB in San Antonio, Texas. Prior to the demonstration, the site groundwater contained about 1 mg/L of PCE and lower amounts of TCE and cis-

DCE, without any detectable VC or ethene. The field test consisted of three recirculation plots, two that served as control plots, and one that was bioaugmented with KB-1™. After equilibration, 13L of KB-1™ were added on day 176. No dechlorination past *cis*-DCE was observed in the control plots, but VC was detected after 52 days in the test plot, and by day 318 ethene was the dominant product. Molecular monitoring showed that the culture had completely colonized the 9.1 meter-long aquifer test plot within 115 days after the one-time injection.

[Harkness, M. R., A. A. Bracco, M. J. Brennan, Jr., K.A. Deweerdt, and J. L. Spivack. 1999. Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene in Dover soil columns. \*Environmental Science and Technology\*, 33:1100-1109.](#)

Soil columns were constructed in support of the Remediation Technologies Development Forum accelerated biodegradation study at Dover Air Force Base to evaluate the impact of amendments on the anaerobic reductive dechlorination of trichloroethene (TCE) in Dover soil. Dechlorination of TCE to *cis*-dichloroethene (*c*-DCE) was observed in the columns using lactate, lactate and methanol, butyrate, glutamate and 1,2-propanediol, or toluene as electron donors, in combination with vitamins and other supplemental nutrients. However, the *c*-DCE formed was not further dechlorinated using any of these amendments. Subsequent inoculation of two columns with a competent, non-native TCE-dechlorinating culture resulted in the dechlorination of TCE to ethene after 30 days. Once the culture was established, dechlorination of TCE to ethene was complete in the first several centimeters of the columns at TCE influent concentrations of 4 mg/L. The culture was also able to dechlorinate TCE to ethene when TCE influent concentrations were increased to 170 mg/L. These results suggest that a critical bacterial population was missing in these soils and that bioaugmentation is an appropriate remedial strategy under such circumstances.

[Maymo-Gatell, X., Y.T. Chien, J.M. Gossett, and S.H. Zinder, 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. \*Science\*, 276: 1568–1571.](#)

Tetrachloroethene is a prominent groundwater pollutant that can be reductively dechlorinated by mixed anaerobic microbial populations to the nontoxic product ethene. Strain 195, a coccoid bacterium that dechlorinates tetrachloroethene to ethene, was isolated and characterized. Growth of strain 195 with H<sub>2</sub> and tetrachloroethene as the electron donor and acceptor pair required extracts from mixed microbial cultures. Growth of strain 195 was resistant to ampicillin and vancomycin; its cell wall did not react with a peptidoglycan-specific lectin and its ultrastructure resembled S-layers of Archaea. Analysis of the 16S ribosomal DNA sequence of strain 195 indicated that it is a eubacterium without close affiliation to any known groups. (Dechlorination inhibited by 200 µg/L chloroform or 3 mg/L 1,1,1-TCE. Studied the factors affecting dechlorination activity by strain 195. Found that the final step, VC to ethene, occurred slowly in a cometabolic process. Demonstrated the ability to dechlorinate at relatively high PCE concentrations, but also showed that other VOCs, notably chloroform and 1,1,1-TCA, caused complete inhibition, at concentrations of 450 µg/L (3.8 µM) and 700 µg/L (5.2

μM), respectively.) (This paper presents the results of efforts to isolate and characterize Dehalococcoides Strain 195, a coccoid bacterium that reductively dechlorinates tetrachloroethene to ethene. Growth of the organism, using H<sub>2</sub> as an electron donor and tetrachloroethene as the electron acceptor, required the addition of a mixed microbial culture.)

[Yang, Y., and P. L. McCarty, 1998. Competition for hydrogen within a chlorinated solvent dehalogenating mixed culture, Environmental Science and Technology, 32\(22\):3591-3597.](#)

Use of an appropriate hydrogen level is necessary to favor dehalogenation of chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), over other hydrogen using processes. This study examined the competition between dehalogenators and other microorganisms occurring in a benzoate-acclimated dehalogenating methanogenic mixed culture. Results show that the dehalogenators competed best against methanogens and homoacetogens when the hydrogen level was maintained between 2 and 11 nM. The 2 nM hydrogen concentration represents the lower threshold value found here for *cis*-1,2-dichloroethene (*cis*-DCE) dehalogenation. The usefulness of this hydrogen range was further confirmed with both batch-fed and continuously-fed reactors. In batch studies, three times more ethene was produced from dehalogenation of *cis*-DCE using propionate than benzoate as electron donor, while benzoate produced three times more methane than propionate. A three times greater hydrogen utilization efficiency for dehalogenation was obtained with a CSTR than with batch reactors when benzoate was used as substrate because a constant hydrogen concentration in the appropriate range could be maintained with the CSTR. These results suggest different approaches that might be used to favor dehalogenators in competition with other microorganisms.

## 6.0 EVIDENCE FOR ENHANCED MASS TRANSFER DURING BIOREMEDIATION

In conjunction with the existing scientific knowledge characterizing microbial degradation of chloroethenes, there are a growing number of laboratory studies characterizing the impacts of enhanced bioremediation processes on the rate of removal of NAPL sources. These studies range in scale from small, well-mixed bioreactors to larger two-dimensional mesocosms although there are some emerging field-scale demonstrations of this technology. The results of laboratory studies suggest that mass transfer enhancement factors as high as six may be achieved in these experimental systems. Reliable mass transfer enhancement factors have yet to be presented in the literature; however, a limited number of modeling studies confirm this result although indicate that there may be some important limitations to the success of this technology. For example, the formation of dechlorinating biomass in close proximity to the NAPL interface can result in significant permeability reductions, potentially limiting electron donor. Biomass itself can serve as an electron donor whereas endogenous decay occurs, a portion of the biomass itself can serve as an electron donor. Competitive donor use by other organisms (e.g., sulfate reducers, methanogens) may further limit effective electron donor delivery.

### 6.1 Laboratory Studies (Simple Reactors, Columns, 2D Mesocosms)

[Carr, C.S., S. Garg, and J.B. Hughes, 2000. Effect of dechlorinating bacteria on the longevity and composition of PCE-containing non-aqueous phase liquids under equilibrium dissolution conditions, \*Environmental Science and Technology\*, 34\(6\):1088-1094.](#)

Enhanced dissolution by biodegradation was investigated in continuous-flow stirred-tank reactors (CFSTRs) bioaugmented with a PCE dechlorinating culture and a model NAPL (PCE in tridecane). Following amendment with formate (10 mM), PCE was dechlorinated to cis-DCE. Over 144 hours, total chlorinated ethenes removal from the biotic reactors was three times greater than the removal from the abiotic reactors. Partitioning of chlorinated products (especially cis-DCE) from the aqueous phase into the NAPL was identified as a mechanism potentially prolonging the longevity of the DNAPL during remediation.

[Cope, N., and J.B. Hughes, 2001. Biologically-enhanced removal of PCE from NAPL source zones, \*Environmental Science and Technology\*, 34\(10\):2014-2021.](#)

The influence of reductive dechlorination on the removal of NAPL (tridecane with 12% w/w containing residual) was investigated using bioaugmented upflow columns flushed with pyruvate (25, 100, and 250 mM) in a nutrient medium. Total chloroethene removal over 75 days was enhanced in the amended columns by up to 6.5 times the chloroethene removal from an abiotic control column with the highest enhancement occurring in the

column with the lowest electron donor dose. Methanogenesis was not a significant competitor for electron donor.

[Seagren, E.A., B.E. Rittman, and A.J. Valocchi, 2002. Bioenhancement of NAPL pool dissolution: experimental evaluation, Journal of Contaminant Hydrology, 55:57-85.](#)

Experiment were conducted using a small one-dimensional columns oriented horizontally. Each column, which contained a small pool of NAPL (toluene mixed with dodecane), was bioaugmented with *Pseudomonas putida* and flushed with aerated mineral medium to promote toluene oxidation. Quasi-steady-state mass balances during abiotic and biotic column runs were used to calculate mass transfer enhancement factors, demonstrating that biodegradation resulted in a statistically significant two-fold increase in toluene dissolution under some experimental conditions. At the highest toluene concentration studied, toluene toxicity appeared to inhibit biodegradation.

[Yang, Y., and P.L. McCarty, 2002. Comparison between donor substrates for biologically enhanced tetrachloroethene DNAPL dissolution, Environmental Science and Technology, 36\(15\):3400-3404.](#)

Three electron donors (pentanol, oleate, and olive oil) were compared in laboratory columns containing PCE DNAPL. In comparison to abiotic dissolution, biodegradation enhanced the rate of DNAPL removal by a factor of about three. The dominant degradation product was cis-DCE, the minimum extent required to enhance PCE mass removal. In the case of the pentanol and oleate-amended columns, detrimental methanogenesis occurred but was minimized by adding a high concentration of dissolved PCE to the column feed solution. Aquifer fouling by biofilm or methane accumulation are possible concerns with the addition of excess electron donor concentration. The use of an electron donor that partitions into DNAPL is proposed.

[Adamson, D.T., J.M. McDade, and J.B. Hughes, 2003. Inoculation of a DNAPL source zone to initiate reductive dechlorination of PCE, Environmental Science and Technology, 37\(11\): 2525-2533.](#)

Bioremediation of a PCE DNAPL source zone was evaluated in a near field-scale model aquifer. The source was bioaugmented and amended with various electron donors, including acetate, lactate, and hydrogen release compound (HRC). Donor addition and bioaugmentation produces sustained PCE dechlorination resulting in cis-DCE and VC accumulation. Minimal ethene formation was observed and the onset of rapid VC production occurred concurrently with the onset of methanogenesis. Although mass transfer enhancement factors were not determined, the initial rate of PCE removal under abiotic conditions was sustained throughout most of biodegradation phase of the study.

## 6.2 Modeling Studies: Include models (software) related to “conceptual model”

[Chu, M., P.K. Kitanidis, and P.L. McCarty, 2003. Effects of biomass accumulation on microbially enhanced dissolution of a PCE pool: a numerical simulation, Journal of Contaminant Hydrology, 65: 79-100.](#)

A modeling study evaluating the effects of PCE biodegradation on the removal of a small PCE pool was completed. Key variables studied included the impact of permeability reductions resulting from biofilm formation (bioclogging) and electron donor concentration. Neglecting bioclogging PCE removal was enhanced proportionally to the electron donor concentration with a four-fold enhancement occurring at the highest electron donor concentration. However, biomass accumulated close (i.e., within several millimeters) to the DNAPL:water interface, forming a low-permeability zone that restricted advective delivery of electron donor to the dechlorinating biomass, a self-limiting condition that decreased PCE dissolution. Under these conditions electron donor delivery to the dechlorinating biomass was primarily through transverse dispersion. The model results were independent of initial biomass concentrations, suggesting that dechlorinating populations will preferentially establish themselves in close proximity to the DNAPL. Since dechlorination occurred so close the DNAPL, the beneficial inhibition of PCE on competing hydrogen-utilization organisms may be minimal.

[Seagren, E.A., B.E. Rittmann and A.J. Valocchi, 1994. Quantitative evaluation of the enhancement of NAPL-pool dissolution by flushing and biodegradation, 28\(5\):853-839.](#)

An analytical solution to the transport equation was used to assess the impacts of flushing velocity and biodegradation on the dissolution of a two-dimensional DNAPL pool under quasi-steady-state conditions. The rate of pool removal by flushing alone is strongly dependent on transverse dispersion and, accordingly, removal rates increase with increasing velocity. At typical groundwater velocities, diffusion is negligible in comparison to transverse dispersivity. Biodegradation enhances dissolution over a wide range of groundwater velocities.

Seagren, E.A., B.E. Rittman, and A.J. Valocchi, 1993. Quantitative evaluation of flushing and biodegradation for enhancing in situ dissolution of nonaqueous-phase liquids, Journal of Contaminant Hydrology, 12:103-132 .

An analytical solution to the transport equation (including rate-limited dissolution and biodegradation) was used to assess the impact of flushing velocity and biodegradation on the dissolution of a one-dimensional domain containing DNAPL. The model provides a framework for understanding the interactions of biodegradation rates, mass transfer kinetics, and groundwater velocity and their effects on DNAPL removal. Conditions favoring rapid DNAPL removal include slow mass transfer and high biodegradation rates.

[Christ, J.A, C.A. Ramsburg, L.M. Abriola, K.D Pennell and F.E. Loffler, 2005. Coupling aggressive mass removal with microbial reductive dechlorination for remediation of](#)

[DNAPL source zones: a review and assessment, Environmental Health Perspectives, 133\(4\):465-477.](#)

Physico-chemical mass removal technologies such as surfactant or cosolvent flushing can be used to aggressively remove DNAPL mass from the subsurface. However, because they involve the injection of potential substrates, these technologies can also promote significant biodegradation of remaining contaminant mass. Both technologies contribute to oxygen depletion and the establishment of reducing conditions, provide reducing equivalents necessary to support reductive dechlorination and improve contaminant bioavailability by facilitating desorption and dissolution. Based on the results of modeling studies, a staged treatment approach including reductive dechlorination following aggressive source treatment can significantly reduce source longevity and long-term risks

### **6.3 Field Studies**

Hood, E.D., D.W. Major, J. Quinn, S. Yoon, A. Gavaskar, and E.A. Edwards, 2006. Demonstration of enhanced bioremediation in a TCE source area, in preparation.

A demonstration of enhanced bioremediation using bioaugmentation and electron donor addition (ethanol) at a test site located at Kennedy Space Centre, Fl. Detailed groundwater monitoring indicated that total chloroethene concentrations in the test plot (including ethene) increases over time, indicating that enhanced removal of sorbed and non-aqueous phase trichloroethene occurred. At the end of the study, ethene was the dominant degradation product. Independent soil sampling within the test plot indicated that more than 99.5% of the initial TCE mass in the test plot was removed.

[Ramsburg, C.A., L.M. Abriola, K.D. Pennell, F.E. Loffler, M. Gamache, B.K. Amos, and E.A. Petrovskis, Stimulated microbial reductive dechlorination following surfactant treatment at the Bachman Road site, Environmental Science and Technology, 38\(22\):5902-5914.](#)

A pilot scale study of surfactant-enhanced remediation was conducted in a homogeneous aquifer. The source area was flushed with 6% solution of Tween 80, removing approximately 19 L of tetrachloroethene. Concentrations of dechlorination daughter products (primarily trichloroethene and cis-1,2,-dichloroethene) increased by two orders of magnitude 450 days after the surfactant flush, indicating that the remaining surfactant had stimulated the native dechlorinating population. Sorption of the Tween 80 to soil formed a zone that slowly released electron donor to stimulate dechlorination.

[Mravik, S.C., R.K. Sillan, A.L. Wood, and G.W. Sewell, 2003. Field evaluation of the solvent extraction residual biotreatment technology, Environmental Science and Technology, 37\(21\):5040-5049.](#)

Cosolvent flushing (95% ethanol) was used to remove tetrachloroethene DNAPL from a sandy source zone. Residual ethanol (2,720 L) was left in the subsurface, providing an

electron donor for dechlorinating microorganisms. Groundwater monitoring performed three years following injection of the cosolvent indicated that cis-1,2-dichloroethene was the major degradation product.

## 7.0 PERFORMANCE MONITORING

The U.S. EPA defines performance monitoring as “the periodic measurement of physical and/or chemical parameters to evaluate whether a remedy is performing as expected” (EPA, 2002).

A well-designed performance assessment program for a DNAPL source zone remediation project should be capable of cost-effectively monitoring progress toward the site-specific treatment goals. A robust performance monitoring program must be based on a valid conceptual model of the site and specific remediation objectives or risk reduction targets.

There are two types of performance monitoring: remedial effectiveness monitoring, and system efficiency monitoring. By collecting performance data in accordance with a well-thought out, systematic plan, decisions concerning continued operation or shut down of a remedial system can be made.

Remedial effectiveness refers to the ability of the system to achieve remediation goals at a given site. Effectiveness is the degree to which a technology application achieves risk reduction goals or response objectives by reducing contaminant mass, concentration, mobility, and/or toxicity while preventing the uncontrolled mobilization or further spread of contaminants. Effectiveness monitoring is typically done after a remedial action has been completed and the project team wishes to verify that the response objectives have been met by comparing post-treatment conditions to baseline conditions. If continued operation is warranted, effectiveness monitoring may be repeated once it appears that the objectives have been met or a decision to cease active remediation is made.

System efficiency monitoring is intended to optimize treatment efficiency by maintaining specific design conditions within the remediation system. These conditions could include appropriate ranges of temperature, pressure, flow rate, pH, dissolved oxygen (DO), ORP, or TOC. This process involves monitoring the reaction zone, including any injection wells, treatment zone monitoring wells, and other subsurface probes. Sample protocols should be specific to the source reduction technology with special attention given to conditions favoring VOC losses (e.g., thermal treatment).

The frequency of efficiency monitoring should be a function of system operation. More frequent monitoring is required earlier in the process; less frequent efficiency monitoring is typically required as the system stabilizes at close-to-optimum conditions. Efficiency monitoring frequency may be as often as weekly to biweekly during the first few months of testing, diminishing to monthly or quarterly for the remainder of the system operation. It is also desirable to schedule monitoring to occur between active source reduction events (e.g., injections) so that the results of field measurements can be used to refine injection or extraction parameters during subsequent events. This reduction of system

efficiency monitoring over time should go hand-in-hand with a shift of resources toward remedial effectiveness monitoring tasks such as soil confirmation sampling, groundwater sampling, or groundwater flux measurements.

Assessing ISB effectiveness is different than for most other DNAPL remediation technologies because of the way the technology is implemented. Whereas most of the aggressive DNAPL remediation technologies discussed in other documents are deployed as one-time, short-duration remedial actions (weeks to months), ISB in DNAPL source zones is typically applied somewhat continuously over a longer time frame (several years).

The most common metric used to assess ISB performance is groundwater concentrations. This metric alone is not generally recommended for assessing the performance of a DNAPL remediation. However, the only parameter measured for most DNAPL performance assessments that use groundwater concentration as a metric is the contaminants that are being remediated. Investigations into the use of alternative metrics such as mass flux are in progress. In contrast, for ISB, several groups of parameters can be monitored through groundwater sampling throughout the operation of an ISB system to assess performance. These include contaminants and degradation products (e.g., PCE, TCE, cis-DCE, trans-DCE, vinyl chloride, ethene, chloride), redox-sensitive parameters (e.g., ORP, DO, ferrous iron, nitrate, sulfate, methane, dissolved hydrogen), electron donor parameters (e.g., chemical oxygen demand, total organic carbon/dissolved organic carbon, speciated electron donors, and volatile fatty acids), biological activity indicators (e.g., carbon dioxide and alkalinity), biological nutrients (e.g., phosphate and nitrogen), and water quality parameters (e.g., temperature, pH, specific conductance). This approach essentially provides a multiple lines of evidence approach to monitoring the performance of ISB.

[National Research Council, 2004. Contaminants in the Subsurface: Source Zone Assessment and Remediation. National Academy Press, Washington, D.C.](#)

This report was prepared by the Committee on Source Removal of Contaminants in the Subsurface of the National Research Council. 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 at their sites.

[Interstate Technology Regulatory Council, 2004. Strategies for Monitoring the Performance of DNAPL Source Zone Remedies, technical report prepared by the Interstate Technology and Regulatory Council's DNAPLs Team.](#)

This document, published by the Interstate Technology & Regulatory Council's DNAPLs Team, is intended for state environmental regulators and others interested in learning about approaches to performance monitoring while implementing various in situ technologies for the treatment of DNAPLs. Despite the ever-increasing number of field applications of DNAPL removal technologies, many unanswered questions remain regarding the effectiveness of these technologies and how best to measure their performance with respect to site-specific remedial objectives. Currently, there is no clear consensus based on objective guidelines as to the best way to evaluate treatment performance and balance performance objectives against site-specific stratigraphy, measurement uncertainties, regulatory acceptance, and cost. The best approach is for site owners, regulators, and stakeholders to understand the options available and the benefits and limitations of each so that informed decisions can be made. The primary purpose of this document is to provide that knowledge base. It presents a number of ways in which the success or failure in treating a DNAPL source zone has been measured and contains several succinct case studies that cover remedial goals and objectives, performance monitoring and verification, and lessons learned.

[U.S. EPA, 2002. Handbook of Groundwater Protection and Cleanup Policies for RCRA Corrective Action. OSWER, EPA/530/R-01/015.](#)

This handbook contains the EPA's latest interpretation of policies on such topics as cleanup goals, the role of groundwater use, point of compliance, source control, and monitored natural attenuation. This Handbook ties 15 different topics together with an overall Groundwater Protection and Cleanup Strategy that emphasizes a phased, results-based approach to cleaning up contaminated groundwater.

## **8.0 EXISTING TECHNICAL GUIDANCE AND DISCUSSION PAPERS ON DNAPL SOURCE REMOVAL**

McCarty, P.L., 1994. An overview of anaerobic transformation of chlorinated solvents in Symposium on Intrinsic Bioremediation of Ground Water: Washington, DC, U.S. Environmental Protection Agency, EPA 540/R/94/515, p. 135-142.

The primary processes by which chlorinated ethenes in groundwater may be biodegraded are aerobic co-metabolism and reductive dechlorination. Aerobic co-metabolism requires both oxygen and suitable electron donor (such as methane, ammonia, or phenol) conditions unlikely to occur frequently in nature. In contrast, reductive dechlorination of tetrachloroethene and trichloroethene occurs frequently under intrinsic although complete dechlorination is associated with conditions favoring methane fermentation.

### [Cleanup Goals Appropriate for DNAPL Source Zones](#)

The U.S. EPA's Ground Water Task Force has developed this discussion paper for input from federal and state regulatory officials, industry groups and other members of the regulated community, as well as environmental and public interest groups. There are differing perspectives on what cleanup goals are appropriate for that portion of the contaminant plume where dense non-aqueous phase liquids (DNAPLs) are present in the subsurface (the DNAPL source zone). The purpose of this paper is to promote dialogue on this issue. It provides a brief background on DNAPLs as a source of contamination, differing stakeholder points of view (based on written or anecdotal input) with respect to challenges posed by DNAPLs, and potential options for addressing these problems (May 2004)

### [The DNAPL Remediation Challenge: Is There a Case for Source Depletion? \(EPA/600/R-03/143\)](#)

This report is the product of an independent expert panel funded by the U.S. EPA Office of Research and Development. Separate dense non-aqueous phase liquids (DNAPL), can serve as persistent sources of dissolved phase contamination and are a major impediment to successful and cost-effective cleanup of sites. Studies have shown that while a significant fraction of the DNAPL mass can be efficiently removed by some technologies in a short period, the efficiency of DNAPL extraction often decays exponentially with increasing mass removal. As a result, there is currently no consensus in the academic, technical and regulatory communities on the ecological or environmental benefits of DNAPL source treatment or on the appropriate metrics for quantifying these benefits. US EPA convened a panel of national and international scientists and practitioners to conduct a critical, independent review of DNAPL remediation issues (December 2003).

### [DNAPL Source Reduction: Facing the Challenge \(DNAPL-2\)](#)

This report was published by the Interstate Technology and Regulatory Council (ITRC). It summarizes current regulatory attitudes toward DNAPL source zone remediation and outlines the pros and cons of partial source removal. Along the way, it challenges assumptions about the infeasibility of removing DNAPLs from certain geological settings where recent advances have made significant source reduction more feasible and cost-effective (April 2002)

[Technical and Regulatory Requirements for Enhanced In Situ Bioremediation of Chlorinated Solvents in Groundwater \(ITRC/ISB-6\).](#)

This document was published by the Interstate Technology and Regulatory Cooperation (ITRC) Council. It was developed to provide guidance to those considering the deployment of enhanced in situ bioremediation of chlorinated solvents in groundwater. It specifically deals with classes of remediation systems designed to remediate or prevent further migration of chlorinated solvents through use of enhancements to the subsurface environment to accelerate solvent biodegradation (December 1998).

["U.S.EPA \(2004\).Handbook of Groundwater Protection and Cleanup Policies for RCRA Corrective Action. Office of Solid Waste and Emergency Response. EPA530-R-04-030, April 2004."](#)

This handbook contains the USEPA's latest interpretation of policies on such topics as cleanup goals, the role of groundwater use, point of compliance, source control, and monitored natural attenuation. It ties 15 different topics together with an overall Groundwater Protection and Cleanup Strategy that emphasizes a phased, results-based approach to cleaning up contaminated groundwater.

[Key OSWER Ground Water Guidances and Reports on Nonaqueous Phase Liquids and Ground Water](#)

This website contains copies of and links to key USEPA Office of Solid Waste and Emergency Response (OSWER) ground water guidance and selected other reports on ground water which are used frequently by Superfund Remedial Project Managers. The documents are organized by topic area.

["USEPA \(2003\). The DNAPL Remediation Challenge: Is There a Case for Source Depletion?," Office of Research and Development, EPA/600/R-03/143, December 2003.](#)

This report contains the findings and recommendations of a panel of national and international scientists and engineers selected by EPA's Office of Research and Development, National Risk Management Research Laboratory (ORD/NRMRL). All members are recognized authorities on the topic of DNAPL remediation. The panel was asked to conduct a critical, independent review of the current state of the science regarding difficulties and benefits associated with remediation of DNAPL source zones and research needs to address this important environmental challenge. The report presents the views of the panel and does not necessarily represent Agency views or policies.

[National Research Council \(2003\). Environmental Cleanup at Navy Facilities: Adaptive Site Management.](#)

This report was prepared to help the Navy's Environmental Restoration Programs address unresolved remediation issues. Among its most important findings is that conventional remediation technologies such as pump-and-treat have been shown to be inadequate in meeting drinking-water-level cleanup standards for many complex sites because of declining contaminant removal rates over time. The report proposes "adaptive site management" for monitored, interpreted, and adjusting remedies in an iterative manner, leading to continual improvements in knowledge and performance. The approach. The report also stresses the need for pilot programs to test new technologies and modifications of existing technologies to improve understanding of the site in parallel with implementation of the chosen remedy.

[USEPA \(2004\). Cleanup Goals Appropriate for DNAPL Source Zones. Discussion Paper.](#)

This discussion paper was developed by EPA's Ground Water Task Force, a workgroup of senior EPA and state regulatory officials, established under the "One Cleanup Program Initiative" of the Office of Solid Waste and Emergency Response (OSWER). The discussion paper discusses differing perspectives on what cleanup goals are appropriate for that portion of the contaminant plume where dense nonaqueous phase liquids (DNAPLs) are present in the subsurface (the DNAPL source zone). It provides a brief background on DNAPLs as a source of contamination, differing 33 stakeholder points of view (based on written or anecdotal input) with respect to challenges posed by DNAPLs, and potential options for addressing these problems. Stakeholders include federal and state regulatory officials, and members of the regulated community, as well as environmental and public interest groups.

[National Research Council \(2004\). Contaminants in the Subsurface: Source Zone Assessment and Remediation. The National Academies Press, Washington, D.C.](#)

This document was prepared by request of the Army It discusses the usefulness and applicability of source remediation as a cleanup strategy, and evaluates what can be accomplished by aggressive technologies in terms of the total contaminant mass removed, risk reduction, and other metrics. Chlorinated solvent DNAPLs are the primary focus of the report; chemical explosives are also considered in depth.

## 9.0 OTHER INTERNET LINKS

### [USEPA OSWER web site for dynamic field activities](#)

US EPA web site defining and promoting the use of dynamic field activities for "Streamlining hazardous waste site activities with real-time data and real-time decisions." Provides links to the guidance document, case studies, field based analytical methods, systematic planning, dynamic work plans, geophysical methods and support software.

### [US EPA Clu-in web site](#)

This web site, hosted by the USEPA Technology Innovation Program, provides a very large database of links to references and technical documents on topics covering all aspects of site characterization. There is a characterization section of the website that provides links for Technology Tools, including Technology Descriptions and Selection Tools, New Technology Initiatives, Methods Information and SOPs, Technology Evaluations, Implementation Software, Technology Vendor and Developer Support. It also provides links for Educational, Policy, and Guidance Materials, including: Brownfields, Systematic Planning, Dynamic Work Plans, Using Field Analytical Methods, Statistics/Sampling Design, Sample Collection and Handling, Site Characterization Case Studies, Modernization Experiences, Topical Papers on Environmental Data Quality. Other links include: Contaminant Focus, a Triad Resource Center, and Publications on Characterization and Monitoring

### [US EPA Technology Innovation Program website](#)

This web site defines its purpose: "to advocate more effective, less costly approaches (i.e. "smarter solutions") by government and industry to assess and clean up contaminated waste sites, soil, and groundwater". It includes numerous references that can be resources for DANPL site characterization.

### [DNAPLs in Groundwater](#)

An educational resource dedicated to Dense Non-Aqueous Phase Liquids, presented by the University of Sheffield.

### [Research on NAPL Source Zones](#)

This website contains links to information on EPA's Ground Water and Ecosystems Restoration Research program within the National Risk Management Research Laboratory.

### [Federal Remediation Technologies Roundtable \(FRTR\)](#)

This website contains technology information compiled from member federal agencies (e.g., USEPA, DoD, DOE). The information includes: FRTR Remediation Case Study Searchable Database containing hundreds of remediation case studies; and Remediation Technology Assessment Reports, a compilation of reports prepared by federal agencies and the ITRC that provide an analysis of remedial technologies based on their use at numerous hazardous waste cleanup sites.

[U.S. Naval Facilities Engineering Service Center, DNAPL Contamination Focus Area](#)

This website contains background to the DNAPL sites, and links to DNAPL characterization and treatment information.

[SERDP/ESTCP DNAPL Source Zone Initiative](#)

This website describes the SERDP and ESTCP projects in the area of DNAPL source zone characterization and remediation.