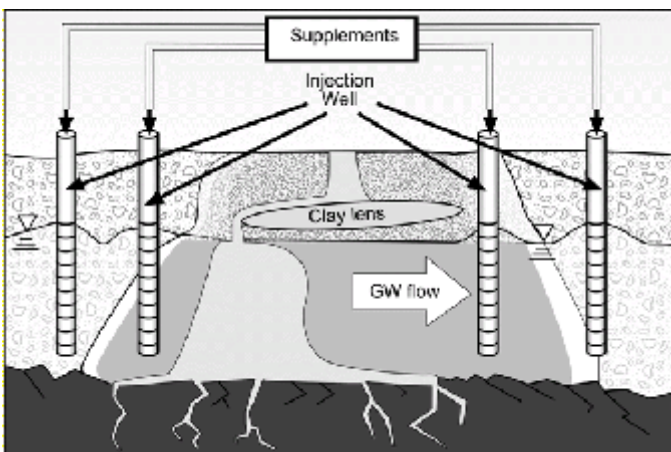
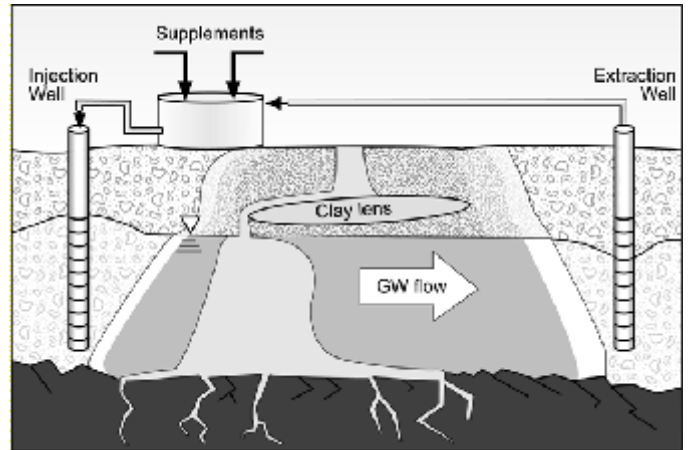
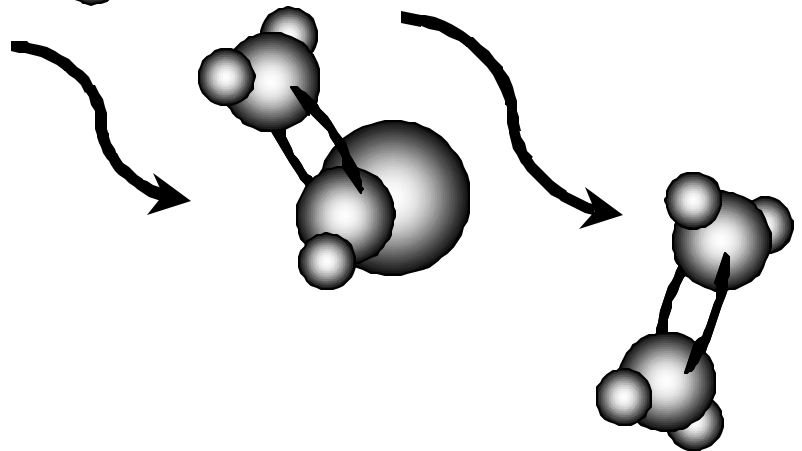
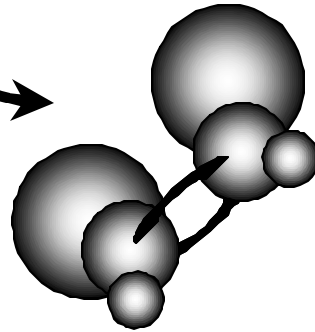
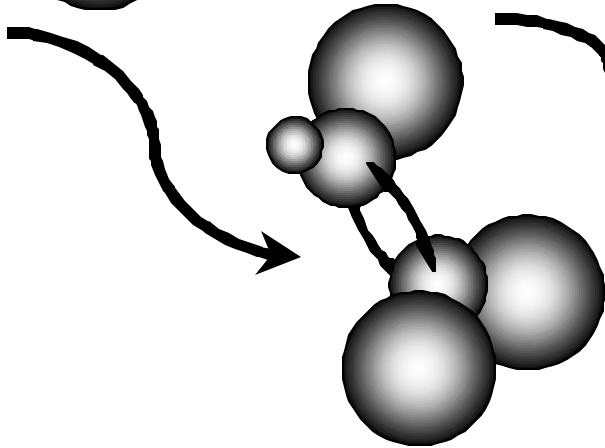
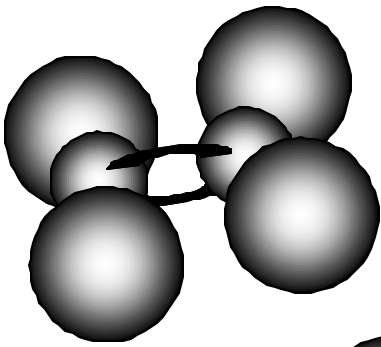




Engineered Approaches to *In Situ* Bioremediation of Chlorinated Solvents: Fundamentals and Field Applications



**ENGINEERED APPROACHES TO *IN SITU* BIOREMEDIATION
OF CHLORINATED SOLVENTS:
FUNDAMENTALS AND FIELD APPLICATIONS**

U.S. Environmental Protection Agency
Office of Solid Waste and Emergency Response
Technology Innovation Office
Washington, DC 20460

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This document was prepared by the U.S. Environmental Protection Agency's Technology Innovation Office under EPA Contract Number 68-W-99-003. Information in this report is derived from a variety of references (including personal communications with experts in the field), some of which have been peer-reviewed. This report has undergone EPA and external review by experts in the field. Key terms used in this report are defined in a glossary, and shown in bold print. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. For more information about this project, please contact: Linda Fiedler, U.S. Environmental Protection Agency, Technology Innovation Office, Ariel Rios Building, 1200 Pennsylvania Avenue, N.W. (MS 5102G), Washington, D.C., 20460; (703) 603-7194; e-mail: fiedler.linda@epa.gov. For technical questions concerning *in situ* bioremediation, please contact: Ann Azadpour-Keeley, U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Subsurface Protection and Remediation Division, P.O. Box # 1198, Ada, Oklahoma, 74820; (580) 436-8890; e-mail: keeley.ann@epa.gov.

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FOREWORD

Halogenated volatile organic compounds, including chlorinated solvents, are the most frequently-occurring type of soil and groundwater contaminant at Superfund and other hazardous waste sites in the United States. The U.S. Environmental Protection Agency (EPA) estimates that, over the next several decades, site owners will spend billions of dollars to clean up these sites. New technologies that are less costly and more effective are needed to accomplish hazardous waste site remediation. As these new and innovative technologies are being developed and used, site managers require information on how they work, their performance to date, and how to evaluate their application at a particular site.

This report provides an overview of the fundamentals and field applications of *in situ* bioremediation to remediate chlorinated solvents in contaminated soil and groundwater. *In situ* treatment is increasingly being selected to remediate sites because it is usually less expensive, and does not require waste extraction or excavation. In addition, *in situ* bioremediation is more publicly acceptable than above-ground technologies because it relies on natural processes to treat contaminants.

This document presents information at a level of detail intended to familiarize federal and state project managers, permit writers, technology users, and contractors with *in situ* bioremediation. The report describes how chlorinated solvents are degraded, how to enhance the process by the addition of various materials and chemicals, design configurations, and the typical steps taken to evaluate technology feasibility at a specific site. It also includes a list of technology vendors and nine case studies of field applications.

It is important to note that this report cannot be used as the sole basis for determining this technology's applicability to a specific site. That decision is based on many factors and must be made on a case-by-case basis. Technology expertise and sometimes treatability studies also are required to make a final remedy decision.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
1.0 INTRODUCTION	1-1
2.0 BIOREMEDIATION MECHANISMS	2-1
2.1 TYPICAL CAHs	2-1
2.2 PHYSICAL AND CHEMICAL PROPERTIES OF CAHs	2-1
2.3 TRANSPORT PROCESSES	2-5
2.4 DEGRADATION MECHANISMS	2-7
2.4.1 Aerobic Oxidation	2-13
2.4.2 Anaerobic Reductive Dechlorination	2-15
2.4.3 Abiotic Degradation Mechanisms	2-17
3.0 <i>IN SITU</i> BIOREMEDIATION TECHNOLOGIES	3-1
3.1 TECHNOLOGIES	3-1
3.2 DESIGN APPROACHES	3-3
3.3 VENDORS OF <i>IN SITU</i> BIOREMEDIATION	3-7
4.0 SELECTION AND IMPLEMENTATION OF <i>IN SITU</i> BIOREMEDIATION TECHNOLOGIES	4-1
4.1 TECHNOLOGY SELECTION AND IMPLEMENTATION	4-1
4.1.1 Site Characteristics	4-1
4.1.2 Site Conditions and Engineering Solutions	4-5
4.1.3 Primary Reactants and Additives Typically Employed	4-6
4.1.4 Treatability (Bench-Scale) Testing	4-7
4.1.5 System Design, Field Testing, and Implementation	4-8
4.2 ADDITIONAL SELECTION FACTORS	4-11
5.0 REFERENCES	5-1
6.0 ADDITIONAL INFORMATION SOURCES	6-1

TABLE OF CONTENTS (continued)

APPENDIX A	CASE STUDIES AND SUMMARIES OF SITES USING <i>IN SITU</i> BIOREMEDIATION TECHNOLOGIES FOR CAHs	A-1
Case Study # 1	Aerobic Degradation Field Demonstration at Moffett Naval Air Station, Mountain View, California	A-15
Case Study # 2	Aerobic Degradation Field Demonstration at Site 19, Edwards Air Force Base, California	A-22
Case Study #3	Methane Enhanced Bioremediation Field Demonstration Using Horizontal Wells at Savannah River Site, Aiken, South Carolina	A-29
Case Study #4	Full-Scale Enhanced Bioremediation at the Texas Gulf Coast Site, Houston, Texas	A-35
Case Study #5	Pilot and Full-Scale Molasses Injection at the Avco Lycoming Superfund Site, Williamsport, Pennsylvania	A-42
Case Study #6	Pilot and Full-Scale Anaerobic <i>In Situ</i> Reactive Zone at an Abandoned Manufacturing Facility, Emeryville, California	A-49
Case Study # 7	Sequential Anaerobic/Aerobic Biodegradation of PCE Field Demonstration at Watertown, Massachusetts	A-56
Case Study # 8	Bioaugmentation (Accelerated Anaerobic Bioremediation) Field Demonstration at Area 6 of the Dover Air Force Base, Dover, Delaware	A-62
Case Study # 9	Cometabolic Bioventing Field Demonstration at Building 719, Dover Air Force Base, Dover, Delaware	A-71

LIST OF EXHIBITS

2-1	CAHs Commonly Identified as Environmental Contaminants	2-2
2-2	Molecular Structures of Common CAHs	2-3
2-3	Chemical and Physical Properties of CAHs	2-4
2-4	Phase Equilibrium Mechanisms and Defining Properties of CAHs	2-5
2-5	Example CAH Subsurface Transport Processes (DNAPL Source)	2-6
2-6	Microbial Biodegradation and Ecology	2-8
2-7	Redox Zones of a Typical Petroleum Plume in an Aerobic Aquifer (Areal View)	2-9
2-8	Selected Information on CAH Degradation Mechanisms	2-10
2-9	Biodegradation Mechanisms Typically Occurring During Enhanced <i>In Situ</i> Bioremediation of CAHs	2-12
2-10	Constituents Involved in Biodegradation Mechanisms for Enhanced <i>In Situ</i> Bioremediation of CAHs	2-12
2-11	Aerobic Oxidation (Direct)	2-13
2-12	Aerobic Oxidation (Cometabolic)	2-14
2-13	Anaerobic Reductive Dechlorination of PCE	2-16
2-14	Example of Hydrolysis Reaction	2-18
2-15	Example of Elimination Reaction	2-18
2-16	Example of Abiotic Reductive Dechlorination	2-18
3-1	Components of <i>In Situ</i> Bioremediation Technology	3-2
3-2	Bioremediation System Configurations	3-5
3-3	<i>In Situ</i> Bioremediation System Configurations	3-6
3-4	Vendors Listed in EPA REACH IT That Provide <i>In Situ</i> Bioremediation Services	3-8
4-1	Typical Selection and Implementation Steps for <i>In Situ</i> Bioremediation	4-2
4-2	Common Site Characterization Parameters Relevant to <i>In Situ</i> Bioremediation	4-3
4-3	Range of Hydrogen Concentrations by Terminal Electron Accepting Process	4-5
4-4	Generally Favorable and Unfavorable Site Conditions for <i>In Situ</i> Bioremediation of CAHs and Typical Engineered Solutions	4-6
4-5	Primary Reactants and Additives Typically Employed for Engineered <i>In Situ</i> Bioremediation of CAHs	4-7
A-1	Summary of Case Studies of <i>In Situ</i> Bioremediation Technology Applications	A-2
A-2	Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings of The Fifth International <i>In Situ</i> and On-Site Bioremediation Symposium	A-4
A-3	Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System	A-10

GLOSSARY OF KEY TERMS

Abiotic	Nonbiological process; also used to refer to nonbiological degradation process.
Adsorption	Removal of a substance from air or water by collecting the substance on the surface of a solid material; process used in pollution control systems such as activated carbon adsorption systems.
Advection	The process of transfer of fluids (vapors or liquid) through a geologic formation in response to a pressure gradient that may be caused by changes in barometric pressure, water table levels, wind fluctuations, or infiltration.
Aerobic	Condition in which oxygen is present; also used to refer to a type of microbe that requires oxygen to live and reproduce.
Aerobic oxidation (cometabolic)	Microbial breakdown of a contaminant during which a contaminant is oxidized by an enzyme or cofactor produced during microbial metabolism of another compound with oxygen. In such a case, the oxidation of the contaminant does not yield any energy or growth benefit for the microbe mediating the reaction.
Aerobic oxidation (direct)	Microbial breakdown of a compound during which the compound serves as an electron donor and as a primary growth substrate by the microbe mediating the reaction. Electrons that are generated by the oxidation of the compound are transferred to oxygen.
Air- or bio-sparging	The process of injecting pressurized air beneath the water table to promote mass transfer of volatile organic compounds out of the groundwater and mass transfer of oxygen into the groundwater.
Anaerobic	Condition in which no oxygen is present; also used to refer to a type of microbe that is able to live and reproduce in the absence of oxygen.
Anaerobic reductive dechlorination (cometabolic)	A biodegradation reaction in which a chlorinated hydrocarbon is reduced by an enzyme or cofactor produced during microbial metabolism of another compound in an environment devoid of oxygen. In such a case, biodegradation of the chlorinated compound does not yield any energy or growth benefit for the microbe mediating the reaction.
Anaerobic reductive dechlorination (direct)	A biodegradation reaction in which bacteria gain energy and grow as one or more chlorine atoms on a chlorinated hydrocarbon are replaced with hydrogen in an environment devoid of oxygen. In the reaction, the chlorinated compound serves as the electron acceptor and hydrogen serves as the direct electron donor. Hydrogen used in this reaction typically is supplied indirectly by the fermentation of organic substrates. The reaction is also referred to as halo-respiration or dehalorespiration.

Bioaugmentation	The addition of microbes to the subsurface where organisms able to degrade specific contaminants are deficient. Microbes may be “seeded” from populations already present at a site and grown in aboveground reactors or from specially cultivated strains of bacteria having known capabilities to degrade specific contaminants.
Bioenergetics	The energy and mass transfer kinetics that are defined by microbial cell metabolism.
Biomass	All the living material in a given area.
Bioremediation	A process by which microorganisms, fungi, and plants degrade pollutant chemicals through use or transformation of the substances.
Capillary forces	Forces that govern fluid flow through small diameter pathways, such as in subsurface soil particles.
Chlorinated aliphatic hydrocarbons (CAHs)	Manmade, chlorine-containing organic compounds widely used as solvents and degreasers in various industries. Typical CAHs include tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC).
Confining layer	Impermeable layer (such as clay) that impedes the vertical migration of groundwater or NAPL.
Degradation	Chemical or biological breakdown of a complex compound into simpler compounds. The breakdown may occur as a result of a single reaction or multiple reactions.
Dense non-aqueous phase liquids (DNAPL)	Chlorinated solvents that are minimally soluble in water, more dense than water, and present in concentrations large enough to form pools of free liquid. DNAPLs tend to sink and accumulate on a non-permeable layer (aquitard) at the bottom of a confined aquifer.
Diffusion	The movement of suspended or dissolved particles (or molecules) from an area of higher concentration to one in which concentrations are lower. This process tends to distribute the particles or molecules more uniformly.
Dispersion	The process by which a substance or chemical spreads and dilutes in flowing groundwater or soil gas.
Electron acceptor	A compound capable of accepting electrons during oxidation-reduction reactions. Microorganisms obtain energy by transferring electrons from electron donors, such as organic compounds (or sometimes reduced inorganic compounds, such as sulfide), to an electron acceptor. Electron acceptors are compounds that are reduced during the process and include oxygen; nitrate; iron (III); manganese (IV); sulfate; carbon dioxide; or, in some cases, chlorinated aliphatic hydrocarbons, such as carbon tetrachloride, PCE, TCE, DCE, and VC.

Electron donor	A compound capable of supplying (giving up) electrons during oxidation-reduction reactions. Microorganisms obtain energy by transferring electrons from electron donors, such as organic compounds (or sometimes reduced inorganic compounds, such as sulfide), to an electron acceptor. Electron donors are compounds that are oxidized during the process and include fuel hydrocarbons and native organic carbon.
Enhanced bioremediation	Bioremediation of organic contaminants by microbes supplemented by increasing the concentration of electron acceptors, electron donors, or nutrients in groundwater, surface water, and soil.
Exogenous bacteria (also called non-indigenous)	Bacteria that have been obtained from a source other than the native site.
Groundwater recirculation treatment system	A closed-loop, hydraulically-contained system based on a design of down-gradient extraction and upgradient injections wells; sometimes referred to as a recirculating treatment cell.
Hydrophobicity	Tendency to repel water.
Metabolic	Having to do with the energy producing processes conducted in cells.
Methanogenic	Referring to the formation of methane by certain anaerobic bacteria during the process of anaerobic fermentation.
Monitored natural attenuation (also known as Passive bioremediation)	Use of natural subsurface processes, such as dilution, volatilization, biodegradation, adsorption, and chemical reactions with subsurface materials to reduce contaminant concentrations.
Nutrients	Elements required for microbial growth. In bioremediation, the term generally refers to elements other than carbon, hydrogen, and oxygen that are required to promote the growth of bacteria. Typical nutrients include nitrogen and phosphorus.
Sorption	The action of soaking up or attracting substances; a general term used to encompass the processes of absorption, adsorption, ion exchange, and chemisorption.
Substrate	A source of energy or molecular building block used by a microorganism to carry out biological processes and reproduce.
Volatilization	The process of transfer of a chemical from the aqueous or liquid phase to the gas phase.

LIST OF ACRONYMS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BFSS	Bioremediation in the Field Search System
CA	Chloroethane
CAHs	Chlorinated aliphatic hydrocarbons
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CF	Chloroform
CFR	Code of Federal Regulations
CM	Chloromethane
CO ₂	Carbon dioxide
CT	Carbon tetrachloride
DCA	Dichloroethane
DCE	Dichloroethene
DNAPL	Dense non-aqueous phase liquid
EPA	U.S. Environmental Protection Agency
EPA REACH IT	EPA REmediation And CHaracterization Innovative Technologies
Fe ₀	Zero-valent iron
Fe ²⁺	Iron (II) ion
GW	Groundwater
H ₂ O ₂	Hydrogen peroxide
HCl	Hydrogen chloride
HRC	Hydrogen release compound
HS ⁻	Hydrogen sulfide ion
LDR	Land Disposal Restrictions
LNAPL	Light non-aqueous phase liquid
MC	Methylene chloride
MNA	Monitored natural attenuation
NAD	Nicotinamide adenine dinucleotide
NAPL	Non-aqueous phase liquid
ORC	Oxygen release compound

PCE	Tetrachloroethene
PRB	Permeable reactive barrier
RCRA	Resource Conservation and Recovery Act
TCA	Trichloroethane
TCE	Trichloroethene
UIC	Underground Injection Control
UST	Underground storage tank
VC	Vinyl chloride

1.0 INTRODUCTION

Halogenated volatile organic compounds (VOCs), including **chlorinated aliphatic hydrocarbons (CAHs)**¹, are the most frequently occurring type of contaminant in soil and groundwater at Superfund and other hazardous waste sites in the United States. The U.S. Environmental Protection Agency (EPA) estimates that cleanup of these sites will cost more than \$45 billion (1996 dollars) over the next several decades (EPA, 1997). Innovative technologies, including *in situ* **bioremediation**, are being developed and implemented in an effort to reduce the cost and time required to clean up those sites. *In situ* bioremediation is increasingly being selected to remediate hazardous waste sites because, when compared to above-ground technologies, it is usually less expensive, does not require waste extraction or excavation, and is more publicly acceptable as it relies on natural processes to treat contaminants.

This report provides an overview of the fundamentals and field applications of *in situ* bioremediation of CAHs in contaminated soil and groundwater, including a summary of currently-available information on the mechanisms and technologies used to implement *in situ* bioremediation. The report is intended to familiarize those involved with hazardous waste site cleanups, including site project managers, contractors, and other technology users, with *in situ* bioremediation. As such, the level of detail included in the report about bioremediation mechanisms, technologies, and implementation is meant to provide basic information about the technology, rather than providing an in depth primer about *in situ* bioremediation. Therefore, the report should be used for informational purposes, but should not be used as the sole basis for determining the use of this technology at a specific site. Such decisions must be made on a case-by-case basis, considering site-specific factors. Information included in this report is not intended to revise EPA policy or guidance concerning site clean up.

Section 2 of this report provides a description of bioremediation mechanisms, including the fate and transport processes and **degradation** mechanisms. Section 3 describes the types of technologies used for *in situ* bioremediation, including approaches used to design remedial systems. Information about vendors of *in situ* bioremediation at sites contaminated with CAHs is also provided. Section 4 discusses the steps involved with the selection and implementation of *in situ* bioremediation as a site remedy, including factors typically considered. The references used in preparing this report are presented in Section 5. Section 6 includes a list of additional information sources providing further detail about *in situ* bioremediation.

Appendix A provides nine case studies of applications of *in situ* bioremediation of CAHs at Superfund and other sites. Of the eight groundwater projects, three involved field demonstrations of **aerobic oxidation (cometabolic)**, three were full-scale projects that used **anaerobic reductive dechlorination**, one was a field demonstration that used a combination of aerobic oxidation (cometabolic) and anaerobic reductive dechlorination, and one was a field demonstration of **bioaugmentation**. The one soil project was a field demonstration of aerobic oxidation (cometabolic).

¹Key terms used in this report are defined in a glossary, and shown in bold print.

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¹Key terms used in this report are defined in a glossary, and shown in bold print.

2.0 BIOREMEDIATION MECHANISMS

This section provides background information about *in situ* **bioremediation** at sites contaminated with **CAHs**, including:

- Typical CAHs (Section 2.1)
- Physical and chemical properties of CAHs (Section 2.2)
- Processes that transport CAHs through the subsurface (Section 2.3)
- Biological and chemical mechanisms that can degrade CAHs (Section 2.4)

More detailed information about the physical and chemical characteristics of CAHs and the subsurface transport processes of CAHs can be found in the references listed in Section 6.0 of this report.

2.1 TYPICAL CAHs

CAHs are manmade organic compounds. They typically are manufactured from naturally occurring hydrocarbon constituents (methane, ethane, and ethene) and chlorine through various processes that substitute one or more hydrogen atoms with a chlorine atom, or selectively dechlorinate chlorinated compounds to a less chlorinated state. CAHs are used in a wide variety of applications, including use as solvents and degreasers and in the manufacturing of raw materials. CAHs include such solvents as tetrachloroethene (PCE), trichloroethene (TCE), carbon tetrachloride (CT), chloroform (CF), and methylene chloride (MC).

Historical management of wastes containing CAHs has resulted in contamination of soil and groundwater, with CAHs present at many contaminated groundwater sites in the United States. TCE is the most prevalent of those contaminants (U.S. Air Force 1998). In addition, CAHs and their **degradation** products, including dichloroethane (DCA), dichloroethene (DCE), and vinyl chloride (VC), tend to persist in the subsurface. Exhibit 2-1 lists the CAHs most commonly identified as environmental contaminants, their abbreviations, their common names, and the types of waste from which they commonly originate. Exhibit 2-2 presents the molecular structures of those CAHs.

2.2 PHYSICAL AND CHEMICAL PROPERTIES OF CAHs

The physical and chemical properties of CAHs govern their fate and transport in the subsurface environment. The number of substituted chlorine atoms on the CAHs directly affects their physical and chemical behavior. As the number of substituted chlorine atoms increases, molecular weight and density generally increase, and vapor pressure and aqueous solubility generally decrease. Exhibit 2-3 lists pertinent physical and chemical data for the CAHs commonly identified as subsurface contaminants.

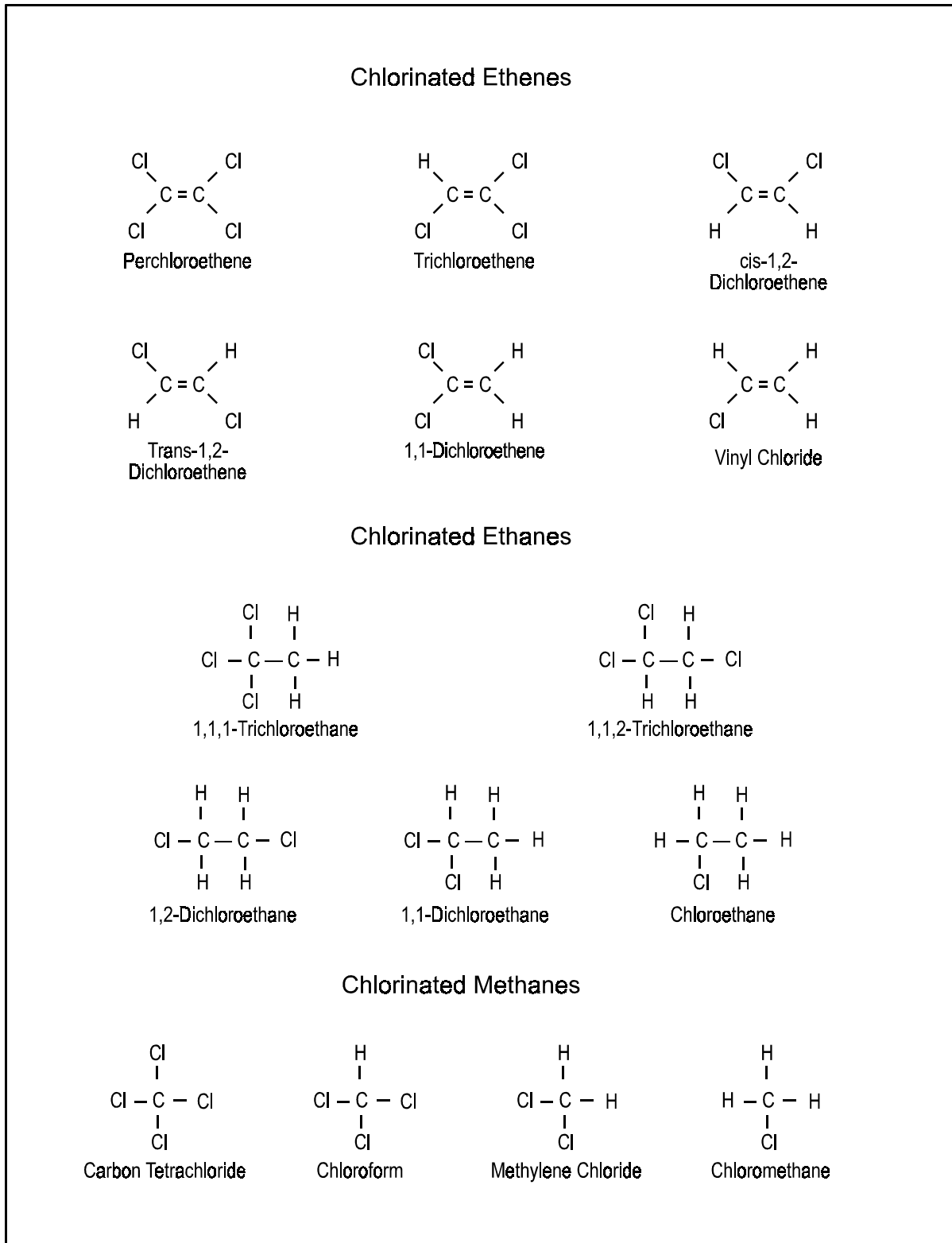
Exhibit 2-1: CAHs Commonly Identified as Environmental Contaminants

Name	Common Name(s)	Abbreviation ¹	Common Waste Sources
CHLORINATED ETHENES			
Tetrachloroethene(-ethylene)	<i>Perchloroethene</i>	PCE	Solvent waste
<i>Trichloroethene(-ethylene)</i>	None	TCE	Solvent waste, degradation product of PCE
<i>cis-1,2-Dichloroethene(-ethylene)</i>	Acetylene dichloride	cis-DCE	Solvent waste, degradation product of PCE and TCE
<i>trans-1,2-Dichloroethene(-ethylene)</i>	Acetylene dichloride	trans-DCE	Solvent waste, degradation product of PCE and TCE
<i>1,1-Dichloroethene(-ethylene)</i>	Vinylidene chloride	1,1-DCE	Solvent waste, degradation product of 1,1,1-TCA
Chloroethene(-ethylene)	<i>Vinyl chloride</i>	VC	Polyvinyl chloride production waste, degradation product of PCE and 1,1,1-TCA
CHLORINATED ETHANES			
<i>1,1,1-Trichloroethane</i>	Methyl chloroform	1,1,1-TCA	Solvent waste
<i>1,1,2-Trichloroethane</i>	Vinyl trichloride	1,1,2-TCA	Solvent waste
<i>1,2-Dichloroethane</i>	Ethylene chloride	1,2-DCA	Solvent waste, degradation product of 1,1,2-TCA
<i>1,1-Dichloroethane</i>	Ethylidene chloride	1,1-DCA	Degradation product of 1,1,1-TCA
<i>Chloroethane</i>	None	CA	Refrigerant waste, tetraethyl lead manufacturing waste, degradation product of 1,1,1-TCA and 1,1,2-TCA
CHLORINATED METHANES			
Tetrachloromethane	<i>Carbon tetrachloride</i>	CT	Solvent waste, fire extinguisher waste
Trichloromethane	<i>Chloroform, methane trichloride</i>	CF	Solvent waste, anesthetic waste, waste degradation product of CT
Dichloromethane	<i>Methylene chloride, methylene dichloride</i>	MC	Solvent waste, degradation product of CT
<i>Chloromethane</i>	Methyl chloride, monochloromethane	CM	Refrigerant waste, degradation product of CT

Notes:
¹Abbreviations are based on the names in bold italic type.

Sources: Sawyer and others 1994; Merck 1989

Exhibit 2-2: Molecular Structures of Common CAHs



Source: Modified from Sawyer and others 1994

Exhibit 2-3: Chemical and Physical Properties of CAHs

Compound	Number of Substituted Chlorine Atoms	Molecular Weight (g/mole) ¹	Liquid Density (g/ml @ 20 °F/4 °C) ¹	Aqueous Solubility (mg/L @ approx. 25 °C) ²	Vapor Pressure (mm Hg @ 25 °C) ²	Log K _{ow} (Octanol/Water Partition Coefficient) ²	Henry's Law Constant (atm-m ³ /mol) ³
Chlorinated Ethenes							
PCE	4	165.8	1.62	150	17.8	2.60	0.0153
TCE	3	131.4	1.46	1,100	57.9	2.38	0.0091
cis-DCE	2	96.9	1.28	3,500	208	0.70	0.0037
trans-DCE	2	96.9	1.28	6,300	324	0.48	0.0072
1,1-DCE	2	96.9	1.21	2,250	600	1.84	0.018
VC	1	62.5	gas	2,670	2,660	1.38	0.315 (5)
Chlorinated Ethanes							
1,1,1-TCA	3	133.4	1.34	1,500	123	2.50	0.008
1,1,2-TCA	3	133.4	1.44	4,500	30	2.47	0.0012
1,2-DCA	2	99.0	1.26	8,520	64	1.48	0.00098
1,1-DCA	2	99.0	1.18	5,500	182	1.79	0.0059
CA	1	64.5	gas	5,700	1,064	1.52 to 2.16 (4)	0.0085
Chlorinated Methanes							
CT	4	153.8	1.59	757	90	2.64	0.0304
CF	3	119.4	1.48	8,200	151	1.97	0.00435
MC	2	84.9	1.33	20,000	362	1.30	0.00268
CM	1	50.5	gas	6,500	4,310	0.95	0.0452 (5)

Notes:

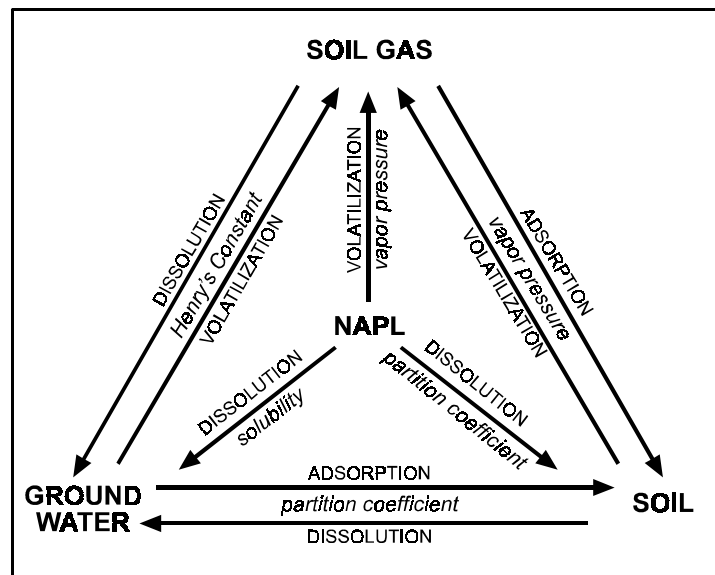
1. Data from Merck Index 1989
2. Data from EPA 1986
3. Data from Gossett 1987
4. Data from EPA 1998
5. Data from EPA 1998a

"Gas" is indicated for liquid density of VC, CA, and CM because they are pure compounds that are gasses under typical environmental conditions.

2.3 TRANSPORT PROCESSES

A CAH released to the subsurface as a pure organic liquid (commonly referred to as non-aqueous phase liquid [NAPL] in the subsurface) will seek phase equilibrium (a condition in which all acting influences are canceled by others, resulting in a stable, balanced, or unchanging system). The CAH will remain as a NAPL, adsorb to soil, dissolve in groundwater, or volatilize into soil gas to the extent defined by the physical and chemical properties of the individual CAH and the subsurface environment. Partition coefficients, which are related to the **hydrophobicity** and aqueous solubility of a CAH, define the extent to which a CAH will partition between NAPL, adsorb to soil, and dissolve in groundwater. The vapor pressure of a CAH defines the extent to which it will partition between NAPL or NAPL adsorbed to soil and the soil gas. CAHs dissolved in groundwater will also partition themselves between the dissolved phase and the vapor phase, as defined by their Henry's Constant. Exhibit 2-4 shows those mechanisms by which CAHs transfer phases in an attempt to reach equilibrium conditions and their related properties.

Exhibit 2-4: Phase Equilibrium Mechanisms and Defining Properties of CAHs



Source: Modified from Huling, S.G. and J.W. Weaver 1991

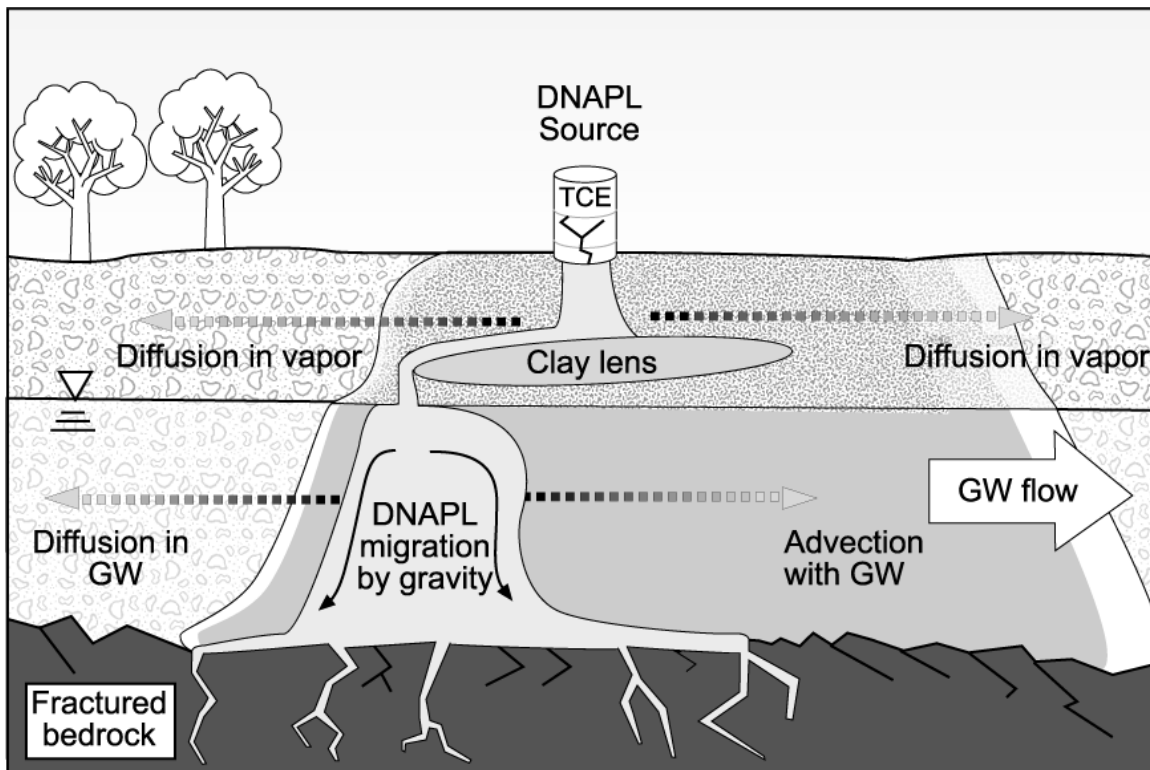
Most of the CAH NAPLs discussed in this report are denser than water (referred to as **dense non-aqueous phase liquids [DNAPLs]**). The exceptions are vinyl chloride, chloroethane, and chloromethane, which are gaseous in their pure phase under standard conditions. DNAPLs tend to sink through both unsaturated and saturated permeable soils until they reach the lowest point on the top of a **confining layer**. NAPLs that are less dense than water (referred to as light non-aqueous phase liquids [LNAPL]) will sink through unsaturated permeable soils and float on the water table, migrating to the lowest water table elevation. In addition, **capillary forces** can trap NAPLs in porous media above or below the water table.

In addition to transferring phases in an attempt to reach equilibrium conditions, CAHs can migrate in the subsurface in their non-aqueous, aqueous, and vapor phases by both active and passive processes. In active processes, such as **advection** and dispersion, CAHs migrate along with the flow of the groundwater or soil gas to which they are partitioned. Passive processes, such as **diffusion**, are the result of concentration gradients, which cause the CAH to seek phase and concentration equilibrium with its surrounding environment. The extent of subsurface migration is a function of the volume of CAH

released, the area over which the release occurs, the duration of the release, and the chemical and physical properties of both the CAH and the subsurface environment.

Typically, releases of CAHs to the groundwater result in the formation of a plume; releases to soil result in subsurface soil contaminated with CAH constituents. In soil, CAHs typically are transported by the flow of DNAPL or diffusion in soil-gas vapor. In groundwater, advective transport (the movement of contaminants by flowing groundwater) is one of the most important processes that affect the transport of dissolved CAHs. In general, the more soluble the compound, the further it will be transported in the subsurface. For example, based on solubility data provided in Exhibit 2-3, MC and CF would be transported more readily in groundwater than PCE and CT. Exhibit 2-5 presents an example of subsurface transport processes.

Exhibit 2-5: Example CAH Subsurface Transport Processes (DNAPL Source)



Source: Modified from Sims and others 1992

2.4 DEGRADATION MECHANISMS

Bioremediation of CAHs can occur through natural mechanisms (intrinsic bioremediation) or by enhancing the natural mechanisms (**enhanced bioremediation**).¹ For a few CAHs (for example, 1,1,1-TCA and CT), degradation also can occur by abiotic (nonbiological) mechanisms. In most systems, biological degradation tends to dominate, depending on the type of contaminant and the groundwater chemistry (EPA 1998). Although a number of biological degradation mechanisms have been identified theoretically and observed on a laboratory scale, the bioremediation mechanisms carried out by bacteria that typically are used for enhanced bioremediation of CAHs generally can be classified into one of the following mechanism categories:

- **Aerobic oxidation (direct and cometabolic)**
- **Anaerobic reductive dechlorination (direct and cometabolic)**

While aerobic oxidation and anaerobic reductive dechlorination can occur naturally under proper conditions, enhancements such as the addition of electron donors, electron acceptors, or nutrients help to provide the proper conditions for aerobic oxidation or anaerobic reductive dechlorination to occur. In general, highly chlorinated CAHs degrade primarily through reductive reactions, while less chlorinated compounds degrade primarily through oxidation (Vogel and others 1987b). Highly chlorinated CAHs are reduced relatively easily because their carbon atoms are highly oxidized. During direct reactions, the microorganism causing the reaction gains energy or grows as the CAH is degraded or oxidized. During cometabolic reactions, the CAH degradation or oxidation is caused by an enzyme or cofactor produced during microbial metabolism of another compound. CAH degradation or oxidation does not yield any energy or growth benefit for the microorganism mediating the cometabolic reaction.

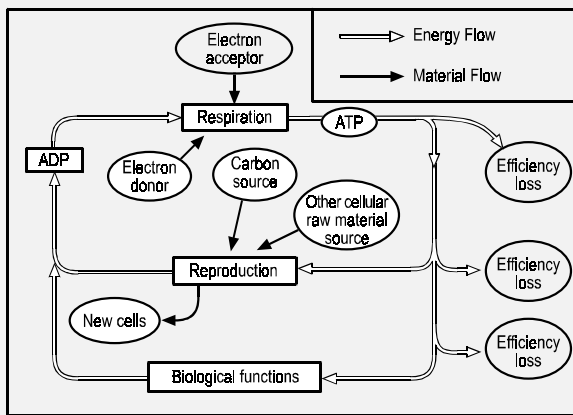
Exhibit 2-6 presents a summary of the nomenclature of microbial biodegradation and ecology. As shown in Exhibit 2-6, biodegradation involves the production of energy in a redox reaction within a bacterial system. This includes respiration and other biological functions needed for cell maintenance and reproduction. Ecology involves the different types of bacteria electron acceptor classes, such as oxygen-, nitrate-, manganese-, iron (III)-, sulfate-, or carbon dioxide-reducing, and their corresponding redox potentials. Redox potentials provide an indication of the relative dominance of the electron acceptor classes.

¹ Although the subject is not covered in this report, CAHs can also be degraded or otherwise removed from soil and groundwater by larger organisms (such as trees), in a process referred to as phytoremediation. References and additional information sources for phytoremediation are listed in Sections 5.0 and 6.0 of this report, respectively.

Exhibit 2-6: Microbial Biodegradation and Ecology

For the most part, the microorganisms that carry out the bioremediation of the CAHs are single-celled procaryotic bacteria. As living organisms, the bacteria require a source of food to survive and propagate. This requirement, or the bioenergetics of a bacterial system, is defined by the thermodynamics of the processes used by the microbes to derive energy and raw materials from substrates and to use them to carry on biological processes and reproduce. The figure below depicts the basic bioenergetics of a typical microbial system.

ADP = adenosine diphosphate



ATP = adenosine triphosphate

In general, the mediating bacteria collect energy in the form of electrons by a chemical reduction-oxidation (redox) reaction (or photosynthesis). The energy is generated in a redox reaction from the transfer of electrons from an electron donor (the organic contaminant in aerobic oxidation (direct)) to an electron acceptor (oxygen in an aerobic reaction). The energy gained is stored as high energy compounds, such as ATP and low-energy compounds, such as nicotinamide adenine dinucleotide (NAD). A portion of the stored energy is used to conduct the biological processes necessary for cell maintenance and reproduction. In addition, cell building-block materials are required in the form of carbon and other nutrients (such as nitrogen and phosphorus). The thermodynamics of a given system defines the energy that is available from a substrate, the energy transfer efficiency losses that will occur, and the portion of the available energy that will be used for reproduction versus the portion that will be used for cell maintenance.

Bacteria generally are categorized by 1) the means by which they derive energy, 2) the type of electron donors they require, or 3) the source of carbon that they require.

Typically, bacteria that are involved in the biodegradation of CAHs in the subsurface are chemotrophs (bacteria that derive their energy from chemical redox reactions) and use organic compounds as electron donors and sources of organic carbon (organoheterotrophs). However, lithotrophs (bacteria that use inorganic electron donors) and autotrophs (bacteria that use carbon dioxide as a carbon source) also may be involved in degradation of CAHs.

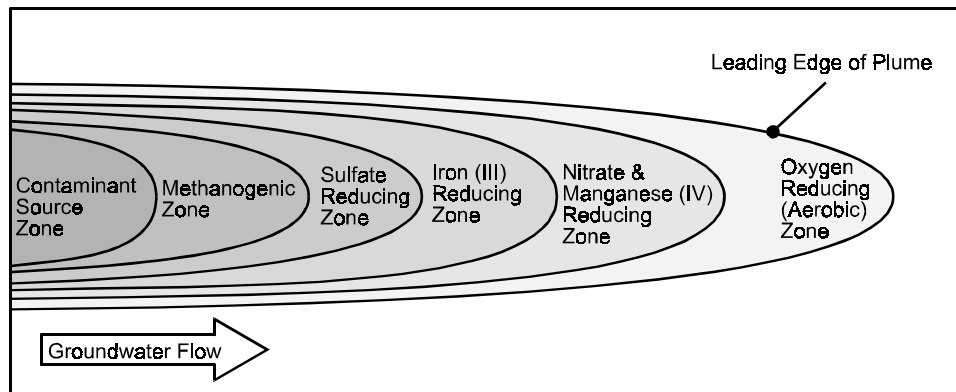
CAH-degrading bacteria are classified further by the electron acceptor that they use, and therefore the type of zone that will dominate in the subsurface (for example, an aerobic zone will dominate when aerobes are present). The typical electron-acceptor classes of bacteria are listed below in the order of those causing the largest energy generation during the redox reaction to those causing the smallest energy generation during the redox reaction. A bacteria electron acceptor class causing a redox reaction generating relatively more energy will dominate over a bacteria electron acceptor class causing a redox reaction generating relatively less energy.

Dominance (as determined by relative energy generation)	Bacteria Electron Acceptor Class	Predominant CAH Biodegradation Mechanism	Approximate Redox Potential (volts) ¹
Most dominant ↓ Least dominant	Oxygen-reducing (aerobes)	Reductive dechlorination	+0.82
	Nitrate-reducing		+0.74
	Manganese (IV)-reducing		+0.52
	Iron (III)-reducing		-0.05
	Sulfate-reducing		-0.22
	Carbon dioxide-reducing (methanotrophs)		-0.24

¹ Standard redox potentials at pH of 7

Exhibit 2-7 shows the redox zones of a typical petroleum plume in an aerobic aquifer, showing the progression from the source area to the edge of the plume. A plume moving with groundwater flow typically will develop distinct redox zones (bacteria will use the electron acceptor that causes the most energy to be generated during the redox reaction when compared with the energy generated from redox reactions using other available electron acceptors). As Exhibit 2-7 shows, once an electron acceptor is depleted, a new redox reaction with the electron acceptor that will result in the next largest generation of energy during the redox reaction will dominate. The dominant redox reaction will determine the type of bacteria that typically will exist in a particular zone and determine the CAH biodegradation mechanisms that may occur.

Exhibit 2-7: Redox Zones of a Typical Petroleum Plume in an Aerobic Aquifer (Areal View)



Source: Modified from Anderson, R.T. and D.R. Lovley 1997

Exhibit 2-8 provides a summary of information available for each degradation mechanism. The degradation mechanisms that typically occur in the biodegradation of each CAH are summarized in Exhibit 2-9, while Exhibit 2-10 presents a summary of the constituents involved in the redox reactions that support each mechanism. Information presented in Exhibit 2-10 was derived from a number of literature sources and from the case studies included in Appendix A of this report.

The remainder of this section presents a more detailed discussion about each of the mechanisms including the specific CAHs that they can degrade, the types of conditions (aerobic or anaerobic) under which they occur, and information about the biology or chemistry of each mechanism.

Exhibit 2-8: Selected Information on CAH Degradation Mechanisms

Degradation Mechanism	CAH	Conditions	Reported Bacteria	Reported Rate Data	Product	Source	
AEROBIC OXIDATION							
Aerobic oxidation (direct)	DCE, VC	Aerobic	Not reported	Observed 2 nd -order Michaelis-Menton kinetics ($V_{max} = 5.1$ and 12.4 $\mu\text{mol/L/D}$ for DCE and VC, respectively)	CO ₂	Bradley and Chapelle 1998	
	DCE, VC, DCA, CA, MC, CM	Aerobic	Not reported	Not reported	CO ₂	RTDF 1997	
Aerobic oxidation (cometabolic)	TCE	Aerobic, electron donor (phenol, toluene, benzene)	<i>Burkholderia cepacia</i> G4, PR1 ₃₀₁	Not reported	CO ₂	Munakata-Marr 1997; McCarty and others 1998	
	TCE	Aerobic, electron donor (toluene)	Not reported	1 st order 0.26-0.4 mol/d	Not reported	Petrovskis and others 1995	
	TCE, DCE, VC, TCA, CF, MC	Aerobic, electron donor (methane, aromatics, ammonia)	Not reported	Not reported	CO ₂	RTDF 1997	
ANAEROBIC REDUCTIVE DECHLORINATION							
Anaerobic reductive dechlorination (dehalorespiration)	PCE, TCE, DCE, VC, DCA	Anaerobic, electron donor (hydrogen or fermentive hydrogen source), relatively low Hydrogen partial pressure	PER-K23, <i>Dehalospirillum multivorans</i> , <i>Dehalobacter restrictus</i> , Dehalococcus ethenogenes	Zero-order dechlorination kinetics for PCE, TCE, cis-DCE and first-order for trans-DCE and VC; total degradation of ~4.6 $\mu\text{mol PCE/mg VSS/day}$	Ethene, ethane	Hollinger 1993; Smatlak 1996; Tandel 1994; Yager 1997; ITRC 2000	
	TCE	Anaerobic, electron donor (lactate, methanol butyrate, glutamate 1,2-propanediol, toluene)	<i>Alcaligenes</i> ; (for example, <i>hydrogenophya</i> present in culture derived from soil)	Michaelis-Menton kinetics	Ethene	Harkness and others 1999	
	PCE, TCE, c-DCE, VC	Anaerobic, electron donor (Hydrogen, propionate or lactate)	Not reported	Not reported	Michaelis-Menton kinetics	Not reported	Ballapragada and others 1997
	PCE	Anaerobic, electron donor (methanol)	Not reported	Not reported	1.24 mg PCE mg /volatile suspended solids (VSS)/d	Not reported	DeStefano 1992
	TCE	Not reported	Not reported	Not reported	1 st order 0.2-4.8 yr ⁻¹	Not reported	Wilson and others, 1996
	DCE	Not reported	Not reported	Not reported	1 st order 0.5-9.4 yr ⁻¹	Not reported	Wilson and others 1996

Exhibit 2-8: Selected Information on CAH Degradation Mechanisms (continued)

Degradation Mechanism	CAH	Conditions	Reported Bacteria	Reported Rate Data	Product	Source
Anaerobic reductive dechlorination (direct) (dehalorespiration) (continued)	VC	Anaerobic, iron-reducing conditions in aquifer	Not reported	Michaelis-Menton $V_{max} = 0.76$ $\mu\text{mol/L-d}$	Not reported	Bradley and Chapelle 1996
	VC	Anaerobic, methanogenic conditions in aquifer	Not reported	Michaelis Menton $V_{max} = 0.19$ $\mu\text{mol/L-d}$	Not reported	Bradley and Chapelle 1997
Anaerobic reductive dechlorination (cometabolic)	PCE, TCE, DCE, VC, DCA	Anaerobic, electron acceptor (nitrate, sulfate), electron donor (hydrogen)	<i>Methanosarcina barkeri</i> , <i>Desulfomonile tiedjei</i>	0.84 and 2.34 nmol PCE/mg protein/day converted to TCE	Ethene, ethane	Fathepure 1987
	PCE, TCE, CT	Anaerobic, electron acceptor (nitrate, sulfate), electron donor (hydrogen)	Methanogens, denitrifiers, sulfate reducers	Not reported	Ethene, methane	Workman 1997; Yager 1997
	CT	Anaerobic, electron acceptor (Fe[III])	<i>Shewanella putrefaciens</i> MR-1	Not reported	CF, MC	Petrovskis and others 1995
ABIOTIC MECHANISMS						
Reductive dechlorination (abiotic)	CT, CF	Anaerobic, metal cofactor, corrinoid, or porphyrin catalyst (reduced vitamin B ₁₂)	Abiotic	Ten-fold rate increase over cometabolic reductive dechlorination	Methane	Workman 1997
Hydrolysis	TCA, CA, CM	Not reported	Abiotic	Not reported	Acetic acid, ethanol	RTDF 1997; EPA 1998
Hydrolysis	TCA	Highly oxidized groundwater	Abiotic	Half life of approximately 2 years	Possibly DCE, acetic acid	McNab and Narasimhen 1994
Elimination	TCA, CA, CM	Not reported	Abiotic	Not reported	DCE	RTDF, 1997; EPA, 1998
Elimination, hydrolysis	TCA	10 °C 15 °C 20 °C	Abiotic	1 st order (at each temperature) 0.058-0.06 yr ⁻¹ 0.137-0.145 yr ⁻¹ 0.31-0.34 yr ⁻¹	DCE, acetic acid	Haag and Mill, 1988; Cline and Delfino 1989; Jeffers and others 1989
Elimination, hydrolysis	TCA	10 °C 15 °C 20 °C	Abiotic	Avg. Half Life (at each temperature) 12 yr 4.9 yr 0.95 yr	DCE, acetic acid	Haag and Mill, 1988; Cline and Delfino, 1989; Jeffers et al., 1989

Exhibit 2-9: Biodegradation Mechanisms Typically Occurring During Enhanced *In Situ* Bioremediation of CAHs

CAH	Aerobic Oxidation		Anaerobic Reductive Dechlorination	
	Direct	Cometabolic	Direct	Cometabolic
CHLORINATED ETHENES				
PCE	X	X	●	●
TCE	X	●	●	●
cis-DCE	X	●	●	●
trans-DCE	X	●	●	●
1,1-DCE	X	●	●	●
VC	●	●	●	●
CHLORINATED ETHANES ¹				
1,1,1-TCA	X	●	X	●
1,2-DCA	●	X	X	●
1,1-DCA	●	X	X	●
CA	X	X	X	X
CHLORINATED METHANES				
CT	X	X	X	●
CF	X	●	X	●
MC	●	●	●	●
CM	●	●	X	X

¹Insufficient information was available for 1,1,2-TCA.

KEY:

- Typically occurring
- X Not typically occurring

Sources: RTDF 1997; ITRC 1998; EPA 1998

Exhibit 2-10: Constituents Involved in Biodegradation Mechanisms for Enhanced *In Situ* Bioremediation of CAHs

Biodegradation Mechanism	Carbon Source	Electron Donor (Reductant)	Electron Acceptor (Oxidant)	Comments
Aerobic oxidation (direct)	CAH	CAH	Oxygen	Only less chlorinated CAHs can be degraded
Aerobic oxidation (cometabolic)	Organic carbon	Cometabolite (e.g., toluene, methane propane)	Oxygen	CAH oxidized by cometabolic mechanism
Anaerobic reductive dechlorination (direct)	Organic carbon or CO ₂	Hydrogen	CAH	Greater chlorinated CAHs are more readily available
Anaerobic reductive dechlorination (cometabolic)	Other organic carbon or CO ₂	Hydrogen	Cometabolic electron acceptor	CAH reductively dechlorinated by cometabolic mechanism

Sources: RTDF 1997; ITRC 1998; EPA 1998

2.4.1 Aerobic Oxidation

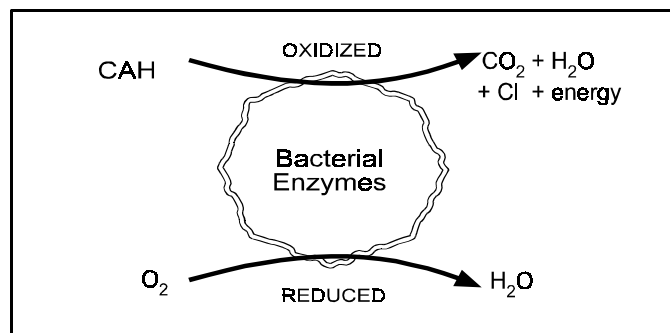
In aerobic zones of the subsurface (zones of the subsurface where oxygen is present), certain CAHs can be oxidized to carbon dioxide, water, and chloride by direct and cometabolic mechanisms (Hartman and DeBont 1992; McCarty and Semprini 1994; Malachowsky and others, 1994; Gerritse and others, 1995; Bielefeld and others 1995; Hopkins and McCarty 1995). Direct mechanisms are more likely to occur with the less chlorinated CAHs (mono- and di-chlorinated). In general, the more chlorinated CAHs can be oxidized by cometabolic mechanisms, but no energy is provided to the organism. Incidental oxidation is caused by enzymes intended to carry out other metabolic functions. Generally, direct oxidation mechanisms degrade CAHs more rapidly than cometabolic mechanisms (McCarty and Semprini 1994) (refer to the following case studies in Appendix A: *Aerobic Degradation in Field Demonstration at Moffett Naval Air Station, Mountain View California [Moffett Field]*; *Aerobic Degradation Field Demonstration at Site 19, Edwards Air Force Base, California [Edwards AFB]*; *Methane Enhanced Bioremediation Using Horizontal Wells at Savannah River Site, Aiken, South Carolina [SRS]*; and *Cometabolic Bioventing at Building 719, Dover Air Force Base, Dover, Delaware [Dover Building 719]*).

Aerobic Oxidation (Direct)

Aerobic oxidation (direct) is the microbial breakdown of a compound in which the compound serves as an electron donor and as a primary growth substrate for the microbe mediating the reaction. Electrons that are generated by the oxidation of the compound are transferred to an electron acceptor such as oxygen.

In addition a microorganism can obtain energy for cell maintenance and growth from the oxidized compound (the compound acts as the reductant). In general, only the less chlorinated CAHs (CAHs with one or two chlorines) can be used directly by microorganisms as electron donors. CAHs that can be oxidized directly under aerobic conditions include DCE, DCA, VC, CA, MC, and CM (Bradley 1998; RTDF 1997; Harkness and others 1999). The CAHs are oxidized into carbon dioxide, water, chlorine, and electrons, in conjunction with the reduction of oxygen to water. Exhibit 2-11 shows an example of aerobic oxidation (direct) of a CAH.

Exhibit 2-11: Aerobic Oxidation (Direct)



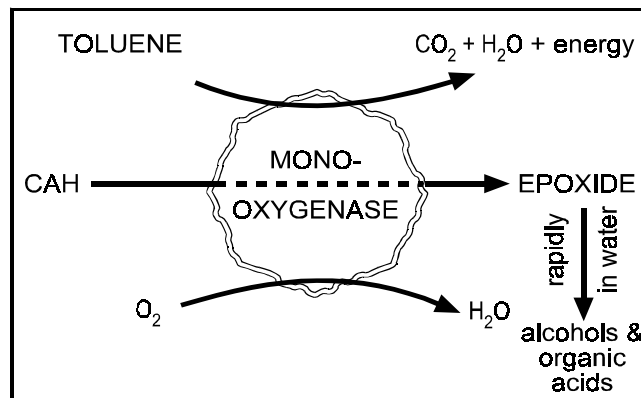
Source: Modified from Hartmans and DeBont 1992

Aerobic Oxidation (Cometabolic)

Aerobic oxidation (cometabolic) is the microbial breakdown of a contaminant in which the contaminant is oxidized incidentally by an enzyme or cofactor produced during microbial metabolism of another compound. In such a case, the oxidation of the contaminant does not yield any energy or growth benefit for the microorganism involved in the reaction.

The CAHs that have been observed to be oxidized cometabolically under aerobic conditions include TCE, DCE, VC, TCA, DCA, CF, and MC (Munakata-Marr 1997; McCarty and others 1998; RTDF 1997; Edwards and Cox 1997; McCarty 1997a; Bradley and Chapelle 1998; Travis and Rosenberg 1997). The electron donors observed in aerobic oxidation (cometabolic) include methane, ethane, ethene, propane, butane, aromatic hydrocarbons (such as toluene and phenol), and ammonia. Under aerobic conditions, a monooxygenase (methane monooxygenase in the case of methanotrophic bacteria) enzyme mediates the electron donation reaction. That reaction has the tendency to convert CAHs into unstable epoxides (Anderson and Lovley 1997). Unstable epoxides degrade rapidly in water to alcohols and fatty acids, which are readily degradable. Exhibit 2-12 shows an example of aerobic oxidation (cometabolic) of a CAH.

Exhibit 2-12: Aerobic Oxidation (Cometabolic)



Source: Modified from McCarty and others 1998

Wilson and Wilson (1985) were the first to observe that the simultaneous addition of methane and oxygen can stimulate biodegradation by aerobic oxidation (cometabolic) of TCE in aquifer material. Subsequently, that approach was tested in the field at Naval Air Station (NAS) Moffett Field, California. Intermittent pulses of oxygen and methane were provided to the subsurface, bringing about the *in situ* stimulation of biodegradation of TCE, c-DCE, and VC in a contaminated aquifer (Semprini and others 1990). The strategy has been applied successfully to biodegradation of CAHs at a variety of other sites (McCarty and others 1991; Travis and Rosenberg 1997).

Although the studies have demonstrated that addition of methane is an effective means of stimulating cometabolic biodegradation of CAHs, additional field studies at the Moffett test site have shown that toluene and phenol can be more effective electron donors than methane in the stimulation of cometabolic biodegradation of TCE, c-DCE, and VC in groundwater (Hopkins and others 1993; Hopkins and McCarty 1995).

2.4.2 Anaerobic Reductive Dechlorination

Under anaerobic conditions, reductive dechlorination mechanisms can effectively biodegrade CAHs. Reductive dechlorination generally involves the sequential replacement of a chlorine atom on a CAH with a hydrogen atom (that is, converting PCE to TCE to DCE, and so on) and has been observed to occur both directly and cometabolically. In anaerobic reductive dechlorination (direct), the mediating bacteria use the CAH directly as an electron acceptor in energy-producing redox reactions. Anaerobic reductive dechlorination (cometabolic) occurs when bacteria incidentally dechlorinate a CAH in the process of using another electron acceptor to generate energy. Reductive dechlorination theoretically is expected to occur under most anaerobic conditions, but has been observed to be most effective under sulfate-reducing and **methanogenic** conditions (EPA 1998). As in the case of aerobic oxidation, the direct mechanisms may biodegrade CAHs faster than cometabolic mechanisms (McCarty and Semprini, 1994) (refer to the following case studies in Appendix A: *Enhanced Bioremediation at the Texas Gulf Coast Site, Houston, Texas*[Texas Gulf Coast]; *Molasses Injection at the Avco Lycoming Superfund Site, Willimasport, Pennsylvania*[Avco Lycoming]; *Anaerobic In Situ Reactive Zone at an Abandoned Manufacturing Facility, Emeryville, California*[Emeryville]; *Sequential Anaerobic/Aerobic Biodegradation of PCE at Watertown, Massachusetts*[Watertown]; and *Bioaugmentation (Accelerated Anaerobic Bioremediation) at Area 6 of the Dover Air Force Base, Dover, Delaware* [Dover Area 6]).

Anaerobic Reductive Dechlorination (Direct)

Anaerobic reductive dechlorination (direct) is a biodegradation reaction in which bacteria gain energy and grow as one or more chlorine atoms on a chlorinated hydrocarbon are replaced with hydrogen (McCarty 1997b; Fennel and others 1997; Mayo-Gatell and others 1997; Gerritse and others 1999). In that reaction, the chlorinated compound serves as the electron acceptor, and hydrogen serves as the direct electron donor (Fennel and others 1997). Hydrogen used in the reaction typically is supplied indirectly through the fermentation of organic substrates. The reaction is also referred to as *halorespiration* or *dehalorespiration* (Gossett and Zinder 1997).

Anaerobic reductive dechlorination (direct) has been observed in anaerobic systems in which PCE, TCE, DCE, VC, and DCA are used directly by a microorganism as an electron acceptor in their energy-producing redox reactions (Neumann and others 1994; Scholz-Muramatsu and others 1995; Freedman and Gossett 1989; Yagi and others 1994; Hollinger and Schumacher 1994; Major and others 1991; McCarty 1997b; Gossett and Zinder 1996; Gerritse and others 1996; DeBruin and others 1992; Maymo-Gatell and others 1997; Sharma and McCarty 1996; Hollinger 1993; Smatlak 1996; Tandol 1994; Yager and others 1997). The mechanism generally results in the sequential reduction of a chlorinated ethene or chlorinated ethane to ethene or ethane. Exhibit 2-13 shows the step-by-step dechlorination of PCE.

The anaerobic reductive dechlorination of the more chlorinated CAHs (PCE and TCE) occurs more readily than the dechlorination of CAHs that already are somewhat reduced (DCE and VC); for that reason, DCE and VC may accumulate in anaerobic environments. It also has been observed that, while VC can be effectively dechlorinated, the presence of PCE in groundwater may inhibit the anaerobic reductive dechlorination of VC (Tandol and others 1994).

VC is more commonly remediated using aerobic mechanisms than anaerobic mechanisms. In anaerobic environments in which VC accumulates, enhanced aerobic bioremediation can be implemented to degrade the VC. Recent studies have demonstrated significant anaerobic oxidation of VC to carbon dioxide under Fe(III)-reducing conditions (Bradley and Chapelle 1998b) and of DCE to VC and VC to carbon dioxide under humic acid-reducing conditions (Bradley and Chapelle 1998a). These studies suggest the possibility of alternative biotransformation mechanisms under anaerobic conditions.

Exhibit 2-13: Anaerobic Reductive Dechlorination of PCE

	$\text{H}_2 \rightarrow \text{HCl}$ PCE → TCE	$\text{H}_2 \rightarrow \text{HCl}$ TCE → DCE	$\text{H}_2 \rightarrow \text{HCl}$ DCE → VC	$\text{H}_2 \rightarrow \text{HCl}$ VC → Ethene	
Carbon oxidation state	+ 2	+ 1	0	- 1	- 2
	Most oxidized → Most reduced				

Source: Modified from DeStephano and others 1992

Hydrogen has been observed to be an important electron donor in anaerobic reductive dechlorination (Fennell and others 1997). The presence of hydrogen establishes a competition between the bacteria that mediate the anaerobic reductive dechlorination (such as *Dehalococcus ethenogenes* and *Dehalospirillum multivorans*) and methanogenic bacteria that also use hydrogen as an electron donor (ITRC 2000). However, it has been observed that the dechlorinating bacteria can survive at a partial pressure of hydrogen ten times lower than that at which the methanogenic bacteria can survive (Smatlak and others 1996), thus providing an opportunity to support the dechlorinating bacteria by providing hydrogen at a slow rate. (Hydrogen addition at a slow rate has been demonstrated with the fermentation of butyric or propanoic acid) (Fennell and others 1997). In addition, in some subsurface environments, competition from nitrate or sulfate-reducing bacteria may limit both methanogenic activity and the extent of anaerobic reductive dechlorination (RTDF 1997).

Studies have shown that anaerobic reduction of CAHs can occur by reductive dechlorination in a variety of environmental conditions (Beeman and others 1994; Semprini and others 1995). A review of the transformation of halogenated compounds has shown that the theoretical maximum redox potential for transformation of PCE to TCE is +580 millivolts and for TCE to DCE is +490 millivolts (Vogel and others 1987). Therefore, the anaerobic reductive dechlorination of the compounds is thermodynamically possible under manganese- or iron-reducing conditions. No peer-reviewed reports of the transformation of PCE to TCE under aerobic conditions were identified. However, the efficiency of the anaerobic dechlorination processes at high redox potential values is limited; efficiency improves as the redox potential decreases.

Pilot studies have been conducted at a variety of sites to examine the feasibility of stimulating *in situ* anaerobic reductive dechlorination by providing to the subsurface simple organic substrates, such as lactate, butyrate, methanol, ethanol, and benzoate (Harkness and others 1999; Freedman and Gossett 1989; Gibson and Sewell 1992; Buchanan and others 1997; Becvar and others 1997; Sewell and others 1998; Litherland and Anderson 1997; Spuij and others 1997).

Anaerobic Reductive Dechlorination (Cometabolic)

Anaerobic reductive dechlorination (cometabolic) is a biodegradation reaction in which a chlorinated hydrocarbon is fortuitously degraded by an enzyme or cofactor produced during microbial metabolism of another compound. In such a case, biodegradation of the chlorinated compound does not appear to yield any energy or growth benefit for the microorganism mediating the reaction (Gossett and Zinder 1997).

Several CAHs have been observed to be reductively dechlorinated by cometabolic mechanisms. In those instances, the enzymes that are intended to mediate the electron-accepting reaction “accidentally” reduce and dehalogenate the CAH. Anaerobic reductive dechlorination (cometabolic) has been observed for PCE, TCE, DCE, VC, DCA, and CT under anaerobic conditions (Fathepure 1987; Workman 1997; Yager and others 1997).

In pilot- and full-scale applications, it is generally difficult to distinguish between direct and cometabolic anaerobic reductive dechlorination reactions. Both biodegradation mechanisms are referred to more generally as anaerobic reductive dechlorination. In laboratory-scale applications, direct and cometabolic anaerobic reductive dechlorination reactions can be distinguished. The role played by anaerobic reductive dechlorination (cometabolic) in relation to anaerobic reductive dechlorination (direct) remains under study.

Anaerobic Reductive Dechlorination Combined with Aerobic Oxidation

Several investigators have suggested that the most efficient bioremediation of CAHs will occur in aquifers that are characterized by an upgradient anaerobic zone and a downgradient aerobic zone (Bouwer 1994; Carter and Jewell 1993; Gerritse and others 1995; Fathepure and others 1987). In the upgradient aerobic zone, anaerobic reductive dechlorination of PCE might degrade to TCE, and eventually to VC. VC could then be degraded by aerobic oxidation (direct) downgradient in the aerobic zone of the CAH plume (the leading-edge fringe of the plume). Stratified redox conditions in the field may provide the best opportunities, other than engineered remedies, for intrinsic biodegradation of CAHs.

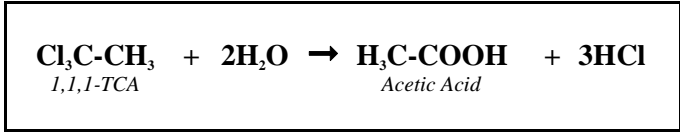
Generally, the substrate requirement for direct metabolism is relatively less than that for cometabolism. In cometabolism, often the amount of primary substrate required is a factor of 100 to 1,000 times the amount of the CAH. In direct metabolism (respiration with only the chlorinated solvent as the electron acceptor), the stoichiometry is much more favorable, and a much smaller amount of supplemental chemical is required (Bouwer 1994).

2.4.3 Abiotic Degradation Mechanisms

Abiotic degradation mechanisms involve chemical reactions to treat CAHs without biological processes. These mechanisms include hydrolysis, elimination, and abiotic reductive dechlorination. In general, the rates of abiotic degradation may be slow relative to biological mechanisms. However, the abiotic mechanisms may play a significant role in the overall remediation of a site at which CAH contamination is present, depending on the specific site conditions (for example, a site at which the contaminant plume is moving slowly) (EPA 1998). Hydrolysis and elimination reactions are generally independent of redox conditions, while abiotic reductive dechlorination is highly dependent on redox conditions.

Hydrolysis is a substitution reaction in which a CAH may react with water to substitute a chlorine atom with a hydroxyl group, producing organic alcohols, acids, or diols, such as the formation of acetic acid from 1,1,1-TCA (Exhibit 2-14). Generally, less chlorinated CAHs are more susceptible to degradation by hydrolysis. Hydrolysis rates have been reported that have half-lives ranging from days for monochlorinated alkanes to thousands of years for tetrachloromethane.

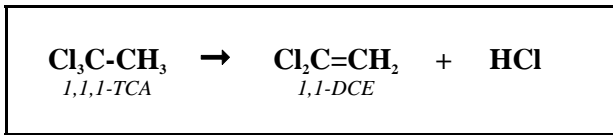
Exhibit 2-14: Example of Hydrolysis Reaction



Hydrolysis is a common transformation mechanism for 1,1,1-TCA, chloroethane, and chloromethane, producing acetate, ethanol, and methanol, respectively (Vogel and McCarty 1987).

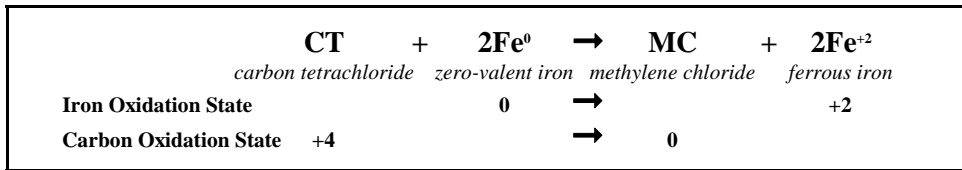
Elimination reactions involve the removal of a hydrogen and a chlorine atom (sometimes referred to as dehydrohalogenation) from a chlorinated alkane, with the formation of the corresponding alkene (Exhibit 2-15). In contrast to hydrolysis reactions, elimination reactions become more effective as the CAHs become more chlorinated. Assuming that elimination rates for monochlorinated CAHs are negligible, the abiotic conversion of TCA to DCE at 20°C has been reported to exhibit relatively rapid first-order kinetics, with a rate constant of approximately $0.04 \pm 0.003 \text{ year}^{-1}$ (Vogel and McCarty 1987).

Exhibit 2-15: Example of Elimination Reaction



Abiotic reductive dechlorination of several CAHs also has been observed (Reinhard and others 1990; Gillham and O’Hannesin 1994; Workman and others 1997). Abiotic reductive dechlorination occurs in the presence of an extremely strong reductant (for example, zero-valent iron or reduced vitamin B₁₂). When the reductant present is sufficiently strong, the more chlorinated (and, therefore, more oxidized) of the CAHs (PCE, TCE, CT, and CF) can be reduced to less chlorinated species without the mediation of bacteria. As in the case of biologically mediated reductive dechlorination, abiotic reductive dechlorination becomes less effective or ineffective for the less chlorinated CAHs (which already are reduced somewhat). Exhibit 2-16 shows the general mechanism of abiotic reductive dechlorination (using zero-valent iron as the reducing agent).

Exhibit 2-16: Example of Abiotic Reductive Dechlorination



3.0 *IN SITU* BIOREMEDIATION TECHNOLOGIES

In situ **bioremediation** technologies are used to enhance the mechanisms that degrade CAHs in contaminated soil and groundwater (discussed in Section 2). Technologies include **bioaugmentation** and the addition of **nutrients**, **electron donors** (substrates such as toluene, propane, and methane), and **electron acceptors** (such as oxygen). Design configurations of *in situ* bioremediation systems include direct injection, groundwater recirculation, installation of permeable reactive barriers (PRBs), and bioventing.

3.1 TECHNOLOGIES

Generally, *in situ* bioremediation technologies employ engineered systems to heighten the effects of naturally occurring **degradation** mechanisms. The engineered systems are designed to include one or more of the following general classes of technologies: the addition of bacteria (bioaugmentation), the addition of nutrients, the addition of electron donors, or the addition of electron acceptors. Each of the technologies is discussed below in more detail. Exhibit 3-1 presents a summary of information about each technology, including an example of how each may be applied, a discussion of the biodegradation mechanisms generally supported by each, a discussion of the typical CAHs targeted through the use of each technology, and a summary of how the enhancement technologies have been applied at the case study sites included in Appendix A of this report.

Bioaugmentation – involves the addition of supplemental microbes to the subsurface where organisms able to degrade specific contaminants are deficient. Microbes may be “seeded” from populations already present at a site and grown in aboveground reactors or from specially cultivated strains of bacteria known to degrade specific contaminants. The application of bioaugmentation technology is highly site-specific and highly dependent on the microbial ecology and physiology of the subsurface (EPA 1998).

Nutrient addition – involves the addition of key biological building blocks, such as nitrogen and phosphorus and other trace nutrients necessary for cell growth. Addition of nutrients generally is applied as a supplement to bioaugmentation or addition of electron donors or electron acceptors, so that concentrations of nutrients in the subsurface do not become a limiting factor for an *in situ* bioremediation application.

Electron donor addition – involves the addition of a substrate that acts as a reductant in the redox reaction used by the CAH-degrading microbe to produce energy. A substrate such as toluene, propane, or methane may be added to act as a cometabolic oxidant, when the CAH also is oxidized. A substrate such as hydrogen, a source of hydrogen, or a hydrogen release compound may be added to act as a direct reductant, when the CAH is reduced.

Electron acceptor addition – involves the addition of oxygen (for **aerobic** mechanisms) or an **anaerobic** oxidant such as nitrate (for anaerobic mechanisms), which is used by the CAH-degrading microbes present in the subsurface.

As Exhibit 3-1 shows, one or more of the technologies were used at several of the case study sites. For example, bioaugmentation was used at *Dover Area 6*, while addition of nutrients was used at *SRS, Texas Gulf Coast, Watertown, and Dover Area 6*. Addition of electron donors, such as toluene, propane, or methane, and an electron acceptor (oxygen) for aerobic cometabolic oxidation were used at the following five sites: *Moffett Field, Edwards AFB, SRS, Watertown, and Dover Building 719*. Addition of an electron donor in the form of a hydrogen source, such as methanol, molasses, or lactate, for anaerobic

Exhibit 3-1: Components of *In Situ* Bioremediation Technology

Component	Example	Biodegradation Mechanisms Supported	Targeted CAHs	Case Study Sites ¹
Bioaugmentation	Seed the subsurface with non-native, CAH-degrading bacteria	Aerobic oxidation (cometabolic and direct)	TCE, DCE, TCA, DCA, CA, CT, CF	None
		Anaerobic reductive dechlorination (cometabolic and direct)	TCA, DCA, CA, CT, CF, CM	Dover Area 6
Addition of Nutrients	Add nitrogen, phosphorus, or other growth factors that may be deficient in the subsurface	Aerobic oxidation (cometabolic and direct)	TCE, DCE, TCA, DCA, CA, CT, CF	SRS; Watertown
		Anaerobic reductive dechlorination (cometabolic and direct)	TCA, DCA, CA, CT, CF, CM	Texas Gulf; Dover Area 6
Addition of Electron Donors	Add a substrate, such as toluene, propane, or methane	Aerobic oxidation (cometabolic)	TCE, DCE, TCA, CF, MC	Moffett Field; Edwards AFB; SRS; Watertown; Dover Building 719
	Add hydrogen, a hydrogen source, or a hydrogen release compound	Anaerobic reductive dechlorination (cometabolic and direct)	PCE, TCE, DCE, VC, TCA, DCA, CA, CT, CF, MC	Texas Gulf; Avco Lycoming; Emeryville; Watertown; Dover Area 6
Addition of Electron Acceptors	Add oxygen by bioventing, biosparging, or adding an oxygen source such as hydrogen peroxide	Aerobic oxidation (direct)	TCE, DCE, VC, TCA, DCA, CA, CE, MC, CM	Moffett Field; Edwards AFB; SRS; Watertown; Dover Building 719
	Add an anaerobic reductant such as nitrate	Anaerobic reductive dechlorination (cometabolic)	PCE, TCE, DCE, VC, DCA, CT	None

Source: ITRC, 1998; Leeson, 1999; Sewell, 1998; U.S. Air Force, 1998

¹ Case studies provided in Appendix A of this report are cited as examples of each of the technologies.

reductive dechlorination was used at the following five sites: *Texas Gulf Coast*, *Avco Lycoming*, *Emeryville*, *Watertown*, and *Dover Area 6* (refer to these case studies in Appendix A).

3.2 DESIGN APPROACHES

The components of *in situ* bioremediation technologies components described above can be implemented in several different general configurations: direct injection, groundwater recirculation, permeable reactive barriers (PRBs), and bioventing. In addition, *in situ* bioremediation may occur naturally, without the application of enhancement technologies. The latter approach is one component of the approach EPA refers to as monitored natural attenuation (MNA)². Because MNA does not use enhancement technologies, it is not discussed in detail in this report.

Exhibit 3-2 includes a summary of the purpose, advantages, and potential limitations of direct injection, groundwater recirculation, PRB, and bioventing systems, described below, and lists the case study sites included in Appendix A at which the configuration was used. Exhibit 3-3 shows the general layouts of the configurations, often referred to as amendment delivery systems. The configurations include use of vertical wells, horizontal wells, and trenches for both injection and extraction of groundwater, or for injection of amendments. Biological, nutrient, electron donor, or electron acceptor amendments are injected in a liquid or a gaseous phase.

Direct injection system - degradation is enhanced through the addition of microbes, nutrients, oxidants, or reductants directly into the aquifer at injection points or directly into the soil. The natural flow of the groundwater generally is not impeded, but is monitored to determine that the degradation of the contaminants and their daughter products is completed within an acceptable distance from the source.

The case study sites at which direct injection into groundwater was used are *SRS*, *Avco Lycoming*, and *Emeryville*. At *SRS*, methane (gas) and air were injected below the water table using a “lower” horizontal well located at a depth of 175 ft below ground surface (bgs). An “upper” horizontal well, located at a depth of 80 ft bgs, was used to extract air and contaminated vapors from the vadose zone. At *Avco Lycoming*, a molasses solution was injected through 20 four-inch diameter wells completed in the overburden. Molasses was added twice each day at various concentrations and rates, as the results of monitoring the system indicated were appropriate.

Groundwater recirculation - extracts contaminated groundwater from the site, adding to or amending the extracted water *ex situ*, and reinjecting the “activated” water to the subsurface, generally upgradient of

² On April 21, 1999, EPA issued a final policy on the use of MNA at Superfund, RCRA corrective action, and underground storage tank (UST) sites (Directive Number 9200.4-17P) available at <http://www.epa.gov/swrust1/directiv/d9200417.htm>. The purpose of the directive was to clarify EPA’s policy regarding the use of MNA for the remediation of contaminated soil and groundwater at sites regulated under the Superfund, RCRA, and UST programs.

As defined in the directive, MNA is the reliance on natural attenuation processes (within the context of a carefully controlled and monitored site cleanup approach) to achieve site-specific remedial objectives within a time frame that is reasonable, compared with that offered by other, more active methods. The processes that are at work in such a remediation approach include a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. Such *in situ* processes include biodegradation; dispersion; dilution; sorption; volatilization; and chemical or biological stabilization, transformation, or destruction of contaminants. Other terms associated with MNA in the literature include “intrinsic remediation,” “intrinsic bioremediation,” “passive bioremediation,” “natural recovery,” and “natural assimilation.”

the contaminated zone. As an alternative, extraction and injection are performed at different elevations in a single well, creating vertical circulation. A groundwater recirculation configuration may be used to provide containment of a plume or to allow the addition of amendments in a more controlled environment.

The case study sites at which groundwater recirculation was used are *Moffett Field, Edwards AFB, Texas Gulf Coast, Watertown, and Dover Area 6*. The project at *Moffett Field* was one of the earliest field demonstrations of *in situ* bioremediation. The *Edwards AFB* project was conducted by the group of researchers who had conducted the *Moffett Field* demonstration, who built upon the results obtained from the earlier project. At *Moffett Field*, groundwater was extracted from one well, amended chemically, and reinjected at another well located 6 meters (m) from the extraction well. The wells, screened in a sand and gravel layer approximately 4 to 6 m bgs, created induced-gradient conditions in the aquifer. At *Edwards AFB*, a two-well recirculation system was constructed; the system created separate bioactive zones in upper and lower aquifers. One well was used to extract groundwater from the lower aquifer, amend it chemically, and reinject the groundwater into the upper aquifer. The other well was used to extract groundwater from the upper aquifer, amend it chemically, and reinject the groundwater into the lower aquifer. The wells were spaced 10 m apart, and screened between approximately 6 and 24 m bgs.

At *Texas Gulf Coast*, the project used a recirculation system that consisted of an alternating series of four extraction and four injection trenches spaced 100 ft apart. Extraction trenches were completed to a depth of approximately 20 to 22 ft bgs, and injection trenches to a depth of approximately 10 ft bgs. At *Watertown*, a recirculation cell that covered a surface area of approximately 10 ft by 20 ft was constructed; the cell had three extraction wells and three injection wells, each screened from 13 to 20 ft bgs. The extraction wells were located at the downgradient end of the cell, and the injection wells at the upgradient end. At *Dover Area 6*, a hydraulically-controlled cell that covered a surface area of approximately 40 ft by 60 ft was constructed; the cell had three extraction wells and three injection wells, each screened from 13 to 20 ft bgs. The field demonstration conducted at *Dover Area 6* included the addition of an aqueous culture of non-indigenous microbes to the groundwater (bioaugmentation).

PRBs - an active bioremediation zone is created by such methods as backfilling a trench with nutrient-, oxidant-, or reductant-rich materials, or by creating a curtain of active bioremediation zone through direct injection or groundwater recirculation at the toe of a plume. PRBs contain a contaminant plume by treating only groundwater that passes through it. PRBs are an emerging design approach for use of *in situ* bioremediation. To date, application of PRBs to *in situ* bioremediation of CAHs has been limited to demonstration tests (ITRC 1997).

None of the case studies included in this report involved the use of a PRB for *in situ* bioremediation of CAHs; however, research has been conducted on the use of such configurations. At the Waterloo Center for Groundwater Research at the University of Waterloo, in Ontario, Canada, a treatment system consisting of a trench (backfilled with sand) was used in a demonstration test at a site with groundwater contaminated with CAHs. In that system, water was extracted from the pore spaces of the wall, amended with nutrients and **substrate**, and reinjected into the wall over a short period of time (a few hours). After reinjection had been completed, the pumps were shut off, and the nutrients were transported out of the wall under natural groundwater flow conditions (as a “slug”). The slug of amended groundwater mixed with surrounding groundwater, and a zone developed in which microorganisms received a continuous supply of the nutrients required to support biodegradation (Devlin and Barker 1994).

Bioventing - the process of aerating soils to stimulate *in situ* biological activity and promote bioremediation. In this process, oxygen is delivered to unsaturated soils by forced air movement (either extraction or injection of air) to increase oxygen concentrations and stimulate biodegradation. Bioventing uses low air flow rates to provide only enough oxygen to sustain microbial activity, with

oxygen most commonly supplied through direct air injection. Bioventing is commonly used for treatment of fuel-contamination in the vadose zone (EPA 1995).

At *Dover Building 719*, an air-sparge blower was used to inject a mixture of air and propane through three injection wells screened to a depth of 10 ft bgs. The *Dover Building 719* site is a field demonstration of bioventing, at which treatment is limited to the soil above the water table.

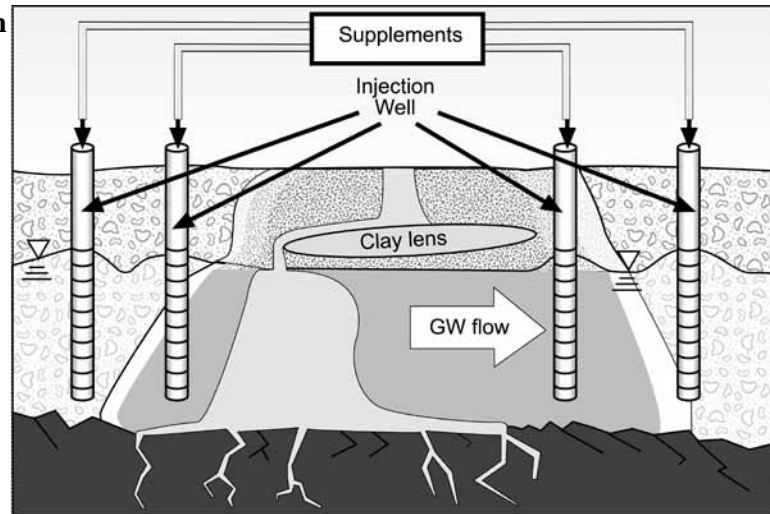
Exhibit 3-2: Bioremediation System Configurations

Configuration	Purpose	Case Study Examples	Applicability/ Advantages	Potential Limitations
Direct injection	To enhance the biodegradation of contaminants in place in soil and groundwater	<i>SRS, Avco Lycoming, Emeryville, Dover Building 719</i>	<ul style="list-style-type: none"> Less aboveground equipment needed than for <i>ex situ</i> systems 	<ul style="list-style-type: none"> Difficult to control dispersion of amendments in aquifer Regulatory concerns about discharge of chemicals to groundwater
Groundwater recirculation	To contain the contaminated groundwater plume and enhance the biodegradation of contaminants in the recirculation area	<i>Moffett Field, Edwards AFB, Texas Gulf Coast, Watertown, Dover Area 6</i>	<ul style="list-style-type: none"> Can provide containment of the plume Allows controlled amendment of groundwater 	<ul style="list-style-type: none"> Reinjection of contaminated groundwater may be complicated because of regulatory concerns Silt build up in recirculation wells reduces effectiveness of systems
PRB	To contain the contaminated groundwater plume and degrade contaminants in groundwater that pass through the barrier	None	<ul style="list-style-type: none"> Less aboveground equipment needed than for <i>ex situ</i> systems 	<ul style="list-style-type: none"> Treats only groundwater that passes through the barrier
Bioventing	To enhance the biodegradation of contaminants in the vadose zone	<i>Dover Building 719</i>	<ul style="list-style-type: none"> Less aboveground equipment needed than for <i>ex situ</i> systems Treats contaminated soil 	<ul style="list-style-type: none"> Bioventing must be coupled with a groundwater treatment technology (such as biosparging) to remediate any contaminated groundwater

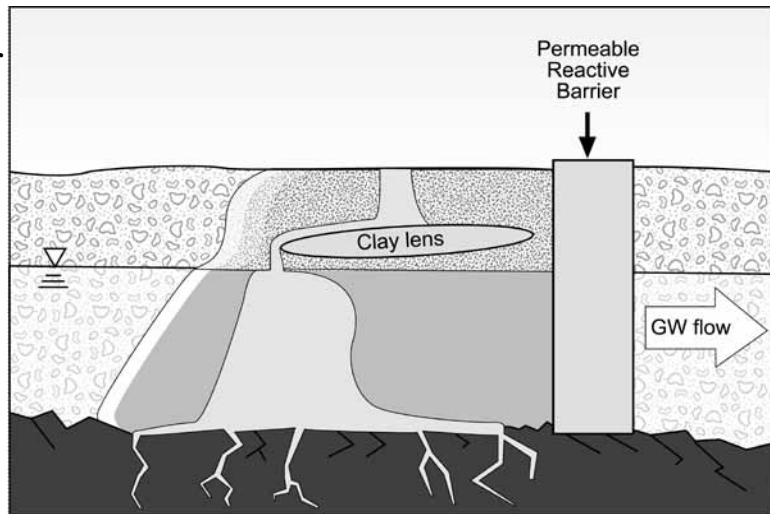
Source: ITRC, 1998; Leeson, 1999; Sewell, 1998; U.S. Air Force, 1998

Exhibit 3-3: *In Situ* Bioremediation System Configurations

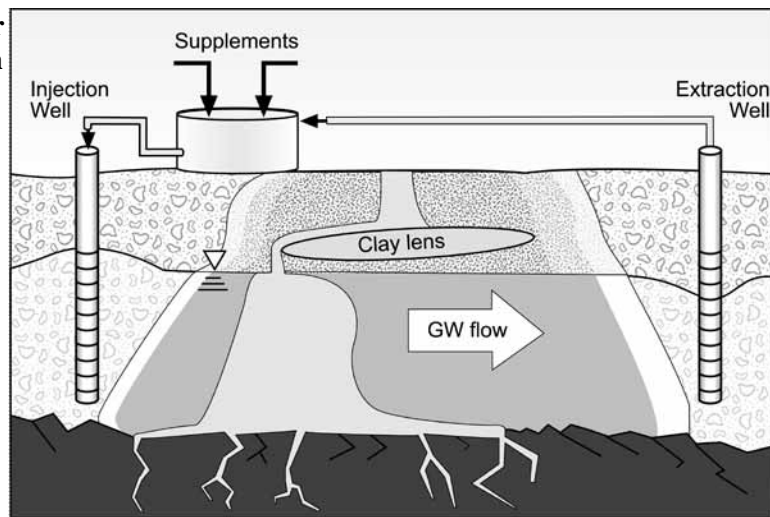
Direct Injection



Permeable Reactive Barrier



Groundwater Recirculation



Source: Modified from USAF 1998

3.3 VENDORS OF *IN SITU* BIOREMEDIATION

This section presents summary information about vendors of *in situ* bioremediation technologies, including contact information and a brief overview of the technology, including information about biodegradation mechanisms and full-scale units. Vendors of *in situ* bioremediation technologies were identified from EPA REACH IT (<www.epareachit.org>), a comprehensive database maintained by EPA about innovative site characterization and remediation technologies. Vendors voluntarily submit information for inclusion in the database. As such, this list of vendors reflects those firms that are currently participating in EPA REACH IT, and may not include all vendors of *in situ* bioremediation technologies. It is also important to note that the information in Exhibit 3-4 is based on information provided by the vendors, and was not independently verified for this report.

As Exhibit 3-4 shows, EPA REACH IT lists 18 *in situ* bioremediation vendors. These vendors offer methods using several types of *in situ* bioremediation mechanisms and technologies at sites contaminated with CAHs:

- **Aerobic oxidation (cometabolic) or anaerobic reductive dechlorination** biodegradation mechanisms
- Direct injection or bioventing **bioremediation** technologies

In addition, more than 130 full-scale units were reported in design, 30 units were being constructed, and 120 units were completed. These units were used to treat media contaminated with CAHs and other contaminants, including petroleum hydrocarbons. Each of the 18 vendors shown in Exhibit 3-4 indicated that a patent is pending for its technology. In addition, 8 vendors have registered technologies, 3 have exclusive licenses, and 3 have patented technologies. To use a patented process, it may be necessary that the user obtain design or construction services directly from the patent holder or purchase a license to provide the technology to others. Interested parties should contact the individual vendors to discuss licensing terms and patent provisions.

The information included in Exhibit 3-4 is current as of June 2000. To search for more current information about *in situ* bioremediation vendors in EPA REACH IT, the following query can be used:

- Technology Type - Bioremediation (*in situ*) - biosparging
Bioremediation (*in situ*) - groundwater
Bioremediation (*in situ*) - lagoon
Bioremediation (*in situ*) - other OR
Bioventing
- AND
- Contaminant Group - All chlorinated ethanes, ethenes, and methanes that were listed individually or in groups under this search criterion were selected for this search

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
ABB Environmental Services, Inc. www.abb.com	Jaret Johnson, P.E. Team Leader Petroleum/Chemical Team Phone: (781) 245-6606 Fax: (781) 246-5060 [no e-mail address identified]	Two-Zone Plume - Interception Treatment Technology	Biological, Nutrient, Oxidant (O), Reductant (O)	2	1	3	<ul style="list-style-type: none"> • Treatment designs include recirculation of groundwater or creation of biological barriers for interception of a plume • Treats BTEX, PAHs, or any contaminant that aerobic bacteria can degraded readily • Removal effectiveness can approach 100 percent 	<p>Anaerobic reductive dechlorination (direct)</p> <p>Aerobic oxidation (cometabolic)</p>	Patent pending
B&S Research, Inc. [No Web page identified]	H.W. Lashmett CEO Phone: (218) 984-3757 Fax: (218) 984-3212 [No e-mail address identified]	Bioremediation (<i>in situ</i>) - groundwater [No trade name given]	Biological, Nutrient	0	6	0	<ul style="list-style-type: none"> • Degrades hydrocarbons, chlorinated solvents, PCBs, fertilizers, pesticides, and other hazardous organic compounds in groundwater and converts contaminants to harmless products • Treatment from surface to 90 feet bgs 	Not specified	Registered trademark; vendor has exclusive license; technology patented; and patent pending

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
Billings & Associates, Inc. [No Web page identified]	Rick Billings Vice President Phone: (505) 345-1116 Fax: (505) 345-1756 [No e-mail address identified]	Groundwater/ Subsurface Volatilization and Ventilation System Remediation Technology	Reductant (O)	100	20	40	<ul style="list-style-type: none"> • Installed rapidly • Uses readily available pumps and pipe valves • Installed with standard drilling equipment • Advantages include use of air to avoid water treatment, ability to manipulate air flows and pressures, and ability to direct air to selected areas • Air emissions can be held within permitted levels by manipulation of flows and use of a biological filter • Has not been applied in treating inorganic wastes 	Aerobic only	Registered trademark and patent pending
Bio-Genesis Technologies www.biogti.com	Victor Coukoulis President Phone: (602) 990-0709 Fax: (602) 990-7745 info@biogti.com	Bioremediation (<i>in situ</i>) - groundwater [No trade name given]	Biological	INP	INP	INP	<ul style="list-style-type: none"> • Treats groundwater contaminated with petroleum hydrocarbons, PAHs, aromatics, alcohols, ketones, phenols, PCBs, solvents, carbohydrates, and pesticides • Advantages include: products are composed of natural biological ingredients that are non-hazardous, no safety equipment is needed, and by-products of the process are non-hazardous 	Not specified	Patent pending

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
Clayton Environmental Consultants [No Web page identified]	John F. Vargas Senior Project Manager Phone: (714) 431-4106 Fax: (714) 825-0685 [No e-mail address identified]	Bioventing [No trade name given]	Nutrient, Reductant (O)	2	0	1	<ul style="list-style-type: none"> • Ease of design and construction • Can be incorporated into a remedial design of soil vapor extraction system • Effective at removing heavier, semivolatile petroleum hydrocarbons such as diesel fuel, fuel oil, kerosene, and JP-4 jet fuel 	Aerobic only	Patent pending
Ecology Technologies International, Inc. [No Web page identified]	Pete Condy President Phone: (916) 939-2397 Fax: (916) 939-2449 PKcondy@aol.com	FYREZYME additive	Biological, Nutrient	1	1	2	<ul style="list-style-type: none"> • Effective in accelerating bioremediation of organic contaminants in an environmentally friendly, scientifically sound, and cost-effective manner • Advantages include: ease of use, permanent solution to contamination, and affordability 	Aerobic only	Registered trademark and patent pending

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
ENSR Consulting and Engineering www.ensr.com	Dan Groher Principal Bioremediation Specialist Phone: (508) 635-9500 Fax: (508) 635-9180 [No e-mail address identified]	Anaerobic Biotransformation with Steam Injection	Nutrient, Oxidant (R)	2	0	2	<ul style="list-style-type: none"> • Combines bioremediation, steam injection, SVE, groundwater extraction, and air-stripping • Effective in removing chlorinated solvents, such as TCA and TCE present as DNAPL; effective for LNAPL • Requires removal of fewer volumes of groundwater, shortens remediation, and therefore reduces costs • Does not treat metals • Injection of substrate and nutrients into groundwater may require a permit 	Anaerobic reduction	Registered trademark and patent pending
		Bioventing [No trade name given]	Nutrient, Reductant (O)	6	3	5	<ul style="list-style-type: none"> • Nutrients may be provided in liquid phase through surface infiltration or in vapor phase through injection by vent wells • Advantages include low cost and demonstrated effectiveness • Low pressure, long-term, low-energy treatment 	Aerobic only	Patent pending
ENVIROGEN, Inc. www.envirogen.com	Michael Shannon Ph.D. Technical Manager Phone: (609) 936-9300 Fax: (609) 936-9221 [No e-mail address identified]	Bioventing [No trade name given]	Nutrient, Reductant (O)	2	0	10	<ul style="list-style-type: none"> • Results in speedier and more cost-effective remediation • Not applicable for metals or nonvolatile or inorganic contaminants 	Aerobic only	Patent pending

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
EOD Technology, Inc. [No Web page identified]	Matt Kaye Director of Marketing and Finance Phone: (423) 690-6061 Fax: (423) 690-6065 eodtmk@aol.com	Bioremediation (<i>in situ</i>) - groundwater/ [no trade name given]	Not specified	INP	INP	INP	<ul style="list-style-type: none"> • Combined with on-site groundwater treatment to enhance the rate of contaminant degradation 	Not specified	Patent pending
In-Situ Fixation, Inc. www.insitufixation.com	Richard P. Murray President Phone: (602) 821-0409 Fax: (602) 786-3184 info@insitufixation.com	Dual Auger System	Biological, Nutrient, Reductant (O)	0	0	1	<ul style="list-style-type: none"> • Increases the quality and acceleration of biodegradation in deep contaminated soils • No excavation is required, and no residues or wastes are generated in the process, since treatment is performed beneath the ground surface 	Aerobic only	Registered trademark; vendor has exclusive license; technology patented; and patent pending
IT Corporation www.itcorporation.com	Dr. Duane Graves Process Development Supervisor Phone: (423) 690-3211 x7418 Fax: (423) 690-9573 [No e-mail address identified]	Restore brand amendments	Nutrient	INP	INP	INP	<ul style="list-style-type: none"> • Information not provided 	Aerobic only	Patent pending

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
<p>Micro-Bac International, Inc. www.micro.com</p> <p>Microbial International</p> <p>Midwest Microbial, L.C.</p>	<p>Terry Nelson Phone: (512) 310-9000 Fax: (512) 310-8800 mail@micro-bac.com</p> <p>Harry Christensen Phone: (714) 666-0110 Fax: (714) 538-5134 micro@webworldinc.com</p> <p>Del Christensen Operating Manager Phone: (402) 493-8880 Fax: (402) 496-9269 [No e-mail address identified]</p>	<p>Bac-Terra Remedial Technology</p> <p>(Three vendors market the same product)</p>	<p>Biological, Nutrient, Oxidant (O), Oxidant (R),</p>	8	0	20	<ul style="list-style-type: none"> • Cost effective, accelerates remedial program, and is adaptable to a broad spectrum of field conditions 	<p>Aerobic and anaerobic mechanisms</p>	<p>Registered trademark; vendor has exclusive license; technology patented; and patent pending</p>
<p>REGENESIS Bioremediation Products www.regenesis.com</p>	<p>Shruti Gohil Assistant Marketing Manager Phone: (949) 443-3136 x27 Fax: (949) 443-3145 orc@genesis.com</p>	<p>Bioremediation (<i>in situ</i>) - groundwater/ oxygen release compound (ORC), hydrogen release compound (HRC)</p>	<p>Reductant (O), ORC, HRC</p>	0	0	2 ³	<ul style="list-style-type: none"> • Information not provided 	<p>Aerobic-ORC, Anaerobic-HRC</p>	<p>Registered trademark; patent pending</p>

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
Richards Laboratories [No Web page identified]	Dr. Sheril D. Burton Senior Microbiologist Phone: (800) 453-1210 Fax: (801) 785-2521 [No e-mail address identified]	Rimlab	Biological, Nutrient, Reductant (O)	2	1	6	<ul style="list-style-type: none"> Treats water saturated with or containing free products of gasoline, diesel fuel, benzene, toluene, monochlorobenzene, dichlorobenzene, crude oil from production wells, hydrocarbons from oil recycling facilities, oil refinery discharges, airport deicing discharges, alcohols from natural gas pipelines, or similar industrial pollutants; a modified consortium degrades TCE Not applicable for treatment of water contaminated with heavy asphaltines Germicidal agents must be neutralized 	Aerobic oxidation (direct)	Patent pending
SBP Technologies, Inc. [No Web page identified]	Director of Business Development Phone: (914) 694-2280 Fax: (914) 694-2286 [No e-mail address identified]	UVB, KGB, BLK	Biological	10	5	16	<ul style="list-style-type: none"> Specially selected microorganisms patented for treatment of water that contains organic wood preservatives, namely creosote and PCP, and chlorinated solvents, especially TCE 	Direct mechanisms only	Patent pending
Terra Vac, Inc. www.terravac.com	Joseph Pezzullo Phone: (609) 371-0070 Fax: (609) 371-9446 [No e-mail address identified]	BIOVAC®	Nutrient, Reductant (O)	0	0	11	<ul style="list-style-type: none"> Significantly more cost-effective than excavation and disposal 	Aerobic oxidation (direct)	Registered trademark and patent pending

Exhibit 3-4: Vendors Listed in EPA REACH IT That Provide *In Situ* Bioremediation Services¹

Vendor	Contact	Trade Name or Technology	Enhanced Bioremediation Amendments ²	# of Full-Scale Units			Technology Performance/ Vendor Claims	Specific Biodegradation Mechanisms Employed	Patent Information
				Design	Construction	Completion			
Waste Stream Technology, Inc. www.wastestream.com	Jim Hyzy Director of Research and Development Phone: (716) 876-5290 Fax: (716) 876-2412 wasstream@aol.com	Bioremediation (<i>in situ</i>) - groundwater [No trade name given]	Biological, Nutrient	0	2	8	<ul style="list-style-type: none"> Costs are reduced by its rapidity, predictability, and technical merit 	Not Specified	Patent pending
Yellowstone Environmental Science (YES), Inc. www.yestech.com	Mary M. Hunter President Phone: (406) 586-2002 Fax: (406) 586-8818 yes@yestech.com	BIOCAT-II (TM)	Biological, Nutrient, Oxidant (O), Oxidant (R), Reductant (O)	INP	INP	INP	<ul style="list-style-type: none"> Treats a variety of wastes, including aromatic hydrocarbons and halogenated hydrocarbons Advantages include: natural pH control and immobilization of metals to protect enriched population of methanogens, conversion of breakdown products of toxic organics to methane that can be used to thermally enhance biotransformations, and elimination of releases of VOC 	Not specified	Registered trademark and patent pending

¹ Information included in this table, including aspects of configuration, number of units, points of contact, list of performance claims, and patent information, was extracted from EPA REACH IT (<<http://www.epareachit.org>>) in June 2000. Information is shown as provided by technology vendors in EPA REACH IT and was not modified for this report.

²

Biological - Bioaugmentation	Oxidant (O) - Oxidant addition for oxidation application
HRC - Hydrogen release compound	Oxidant (R) - Oxidant addition for reductive dechlorination application
Nutrient - Nutrient addition	ORC - Oxygen release compound
INP - Information Not Provided	Reductant (O) - Reductant addition for oxidation application (oxygen unless otherwise specified)
	Reductant (R) - Reductant addition for reductive dechlorination (cometabolic)

³ At an EPA Region 9 meeting on September 23, 1999, Regensis Bioremediation Products claimed to have used its ORC product at more than 5,000 sites and its HRC product at more than 100 sites.

4.0 SELECTION AND IMPLEMENTATION OF *IN SITU* BIOREMEDIATION TECHNOLOGIES

This section discusses the general steps involved in selecting and implementing *in situ* bioremediation technologies, and additional factors relevant to using this technology. As discussed in Section 1, this report is intended to familiarize those involved with hazardous waste site cleanups, including site project managers, contractors, and other technology users, with *in situ* bioremediation. As such, the level of detail included in the report about bioremediation mechanisms, technologies, and implementation is meant to provide basic information about *in situ* bioremediation. Therefore, the report should be used for informational purposes, but should not be used as the sole basis for determining the use of this technology at a specific site. Decisions about the use of in-site bioremediation must be made on a case-by-case basis, considering site-specific factors. Information included in this report is not intended to revise EPA policy or guidance concerning site clean up.

4.1 TECHNOLOGY SELECTION AND IMPLEMENTATION

The steps typically followed in the selection and implementation of an *in situ* bioremediation system at a site contaminated with CAHs are generally the same as the steps taken to implement other types of remedial systems. However, special attention is typically given to identifying the **degradation** mechanisms that may be used to remediate the site and the enhancement technologies that could be beneficial for use at the site. Exhibit 4-1 shows the typical steps in selection and implementation of *in situ* bioremediation, which are:

- Evaluate site characteristics
- Identify general site conditions and engineering solutions
- Identify primary reactants and possible additives
- Perform treatability (bench-scale) testing
- Perform system design, field testing, and implementation

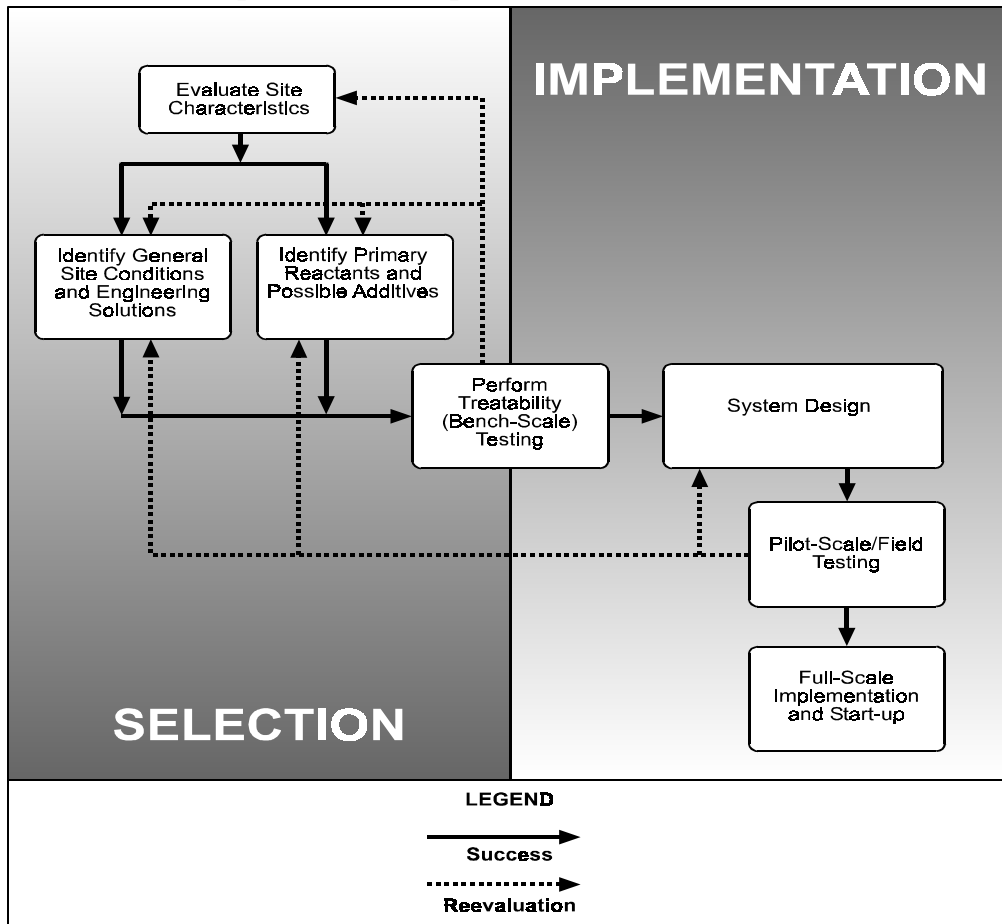
4.1.1 Site Characteristics

Site characteristics relevant to *in situ* bioremediation of CAHs include physical, chemical, and biological parameters. Exhibit 4-2 summarizes the parameters that are commonly evaluated for a site where *in situ* bioremediation of CAHs is being considered. These parameters are also relevant to the design and implementation of the technology, as discussed later in this section.

Physical Parameters - Physical parameters determine how and at what rate liquids and gases move through soils, aquifers, and other geologic units. Common physical parameters that are relevant to *in situ* bioremediation include porosity, hydraulic conductivity, and hydraulic gradient of the geologic unit, and the organic and moisture content of the soil. Because these parameters affect the flow rate of fluids, they also are considered in determining the delivery method for any amendments that are used.

Hydrogeologic studies help determine information about several physical parameters such as groundwater flow, and contaminant fate and transport, and might include aquifer parameter testing, tracer tests, and hydrogeologic flow and transport modeling. Aquifer parameter tests include either slug tests or down hole velocity measurements. Tracer tests have been conducted using constituents such as sodium bromide, added at 100 times its detection limits. According to ITRC, the most commonly used flow model is the U.S. Geological Survey model MODFLOW, which is often coupled with transport models such as RT3D or MT3D, and a particle tracking module such as MODPATH. (ITRC, 1998)

Exhibit 4-1: Typical Selection and Implementation Steps for *In Situ* Bioremediation



Chemical Parameters - Chemical parameters, along with biological parameters, affect the type of degradation mechanisms that are likely to occur and the rate of degradation. Common chemical parameters that are relevant to *in situ* bioremediation include concentrations of CAHs and daughter products, oxygen content, pH, redox potential, concentrations of electron donors and acceptors, and nutrient concentrations. Such parameters provide information about the baseline contamination at the site, whether the natural conditions at the site are aerobic or anaerobic, whether sufficient electron donors and acceptors are present to support biodegradation, and whether and how much intrinsic biodegradation (without enhancements) may be occurring at the site. Several of these parameters are discussed in more detail below.

CAH concentrations affect the specific degradation mechanisms that may be occurring at the site and the substrate levels for direct degradation. In addition, the presence of co-contaminants may affect biodegradation. For example, organic compounds such as toluene, methane, or phenol may augment the performance by providing a substrate for oxygen depletion or for cometabolic degradation. Alternatively, biodegradation may be limited by high concentrations of metals or other toxic compounds that may inhibit microbial activity.

Exhibit 4-2: Common Site Characterization Parameters Relevant to *In Situ* Bioremediation

Parameter	Relevance	Typical Measurement Method	Typical Units
PHYSICAL PARAMETERS			
Soil porosity	To determine the extent to which soil gas diffusion (natural or forced) can take place	Soil sampling	Percent
Hydraulic conductivity	To determine the potential rate of groundwater flow through soil or in an aquifer	Aquifer testing; permeability testing	Length per time
Hydraulic gradient	To determine the speed at which groundwater travels (when combined with hydraulic conductivity)	Water level measurements	Elevation difference per length
Organic content of soil	To determine the extent to which contaminants may adsorb to soil, rather than migrate with groundwater; provide a source of carbon for biodegradation reactions	Volatile solids analysis	Percent by weight
Moisture content of soil	To determine whether sufficient moisture is present in soil to support degradation processes	Dry weight measurement	Percent by weight of water
CHEMICAL PARAMETERS			
CAH concentrations	To determine the baseline level of contamination, specific degradation mechanisms that may be applicable, and substrate levels for direct degradation	Gas chromatography with flame ionization detector or mass spectrophotometric methods	Amount of analyte per volume of water (e.g., $\mu\text{g/L}$) or per mass of soil (e.g., mg/kg)
Concentrations of CAH degradation products (that is, less chlorinated CAHs or non-chlorinated products)	To provide a measure of degradation mechanisms taking place naturally	Gas chromatography with flame ionization detector or mass spectrophotometric methods	Amount of analyte per volume of water (e.g., $\mu\text{g/L}$) or per mass of soil (e.g., mg/kg)
Concentrations of organic electron donors (for example, toluene, methane, phenol, or organic acids)	To determine the potential for cometabolic degradation mechanisms without enhancements	Gas chromatography with flame ionization detector or mass spectrophotometric methods; oxygen uptake analysis	Amount of analyte per volume of water (e.g., $\mu\text{g/L}$) or per mass of soil (e.g., mg/kg)
Concentrations of inorganic electron donors (for example, hydrogen, iron, or ammonia)	To determine the potential for degradation mechanisms without enhancements	Direct electrode; wet chemistry methods; atomic adsorption methods	Amount of analyte per volume of water (e.g., $\mu\text{g/L}$) or per mass of soil (e.g., mg/kg)
Oxygen content	To determine whether aerobic or anaerobic conditions are present	Direct electrode	Percent oxygen or concentration by volume

Exhibit 4-2: Common Site Characterization Parameters Relevant to *In Situ* Bioremediation

Parameter	Relevance	Typical Measurement Method	Typical Units
CHEMICAL PARAMETERS (continued)			
Redox potential	To determine the approximate size and location of a plume; determine whether aerobic or anaerobic conditions are present; identify microbes that are likely to be present	Direct electrode	Volts relative to standard electrode (more negative is a more reducing environment and more positive is a more oxidizing environment)
Concentrations of other electron acceptors (for example, nitrate, sulfate, carbon dioxide)	To determine whether electron acceptors are sufficient for aerobic degradation of CAHs, or may cause unwanted competition in anaerobic dehalogenation processes	Direct electrode; wet chemistry methods; atomic adsorption methods	Amount of analyte per volume of water (e.g., µg/L) or per mass of soil (e.g., mg/kg)
Concentrations of nutrients (for example, boron, calcium, magnesium, manganese, nitrogen, potassium, or phosphorus)	To determine whether nutrient levels are sufficient for microbial activity or whether nutrient addition is needed	Wet chemistry methods; atomic adsorption methods	Amount of analyte per volume of water (e.g., µg/L) or per mass of soil (e.g., mg/kg)
pH	To determine whether conditions are most beneficial for microbial growth	Direct electrode	Standard pH units
BIOLOGICAL PARAMETERS			
Presence and concentration of non-specific microbes	To estimate microbial abundance	Direct microscopic analysis; electronic particle counting; volatile suspended solids analysis; total kjeldahl nitrogen analysis; protein analysis	Concentration of total solid organic material in terms of total organic carbon
Presence and concentration of specific microbes	To determine the presence and concentration of targeted type of organism	Heterotrophic plate count; MPN tube counts	Count of specific indigenous bacteria per volume of culture media
Microbial activity	To quantify the rate of activity of targeted organisms	Oxygen uptake rate analysis; dehydrogenase activity analysis	Amount of oxygen consumed during a specified time period

Sources: Gossett, *Microbiology for Environmental Engineers* 1997; EPA 1998

The presence, concentration, and distribution of daughter products often is used as an indicator that biodegradation may be taking place *in situ*. For the CAH TCE, an increase in concentration of DCE in groundwater (a daughter product of TCE), along with a decrease in TCE concentrations can be used as an indicator that biodegradation is occurring. The presence of VC, a degradation product of DCE, and ethene, a degradation product of VC, can be used as indicators of the biodegradation process.

The concentration of dissolved hydrogen in the subsurface can be used as an indicator of the type of terminal electron-accepting process is occurring. Exhibit 4-3 provides data on the hydrogen concentration for five processes - denitrification, iron (III) reduction, sulfate reduction, reductive dechlorination, and methanogenesis. The type of terminal electron-accepting process helps to determine the dominant types of microbes present at a site.

Exhibit 4-3: Hydrogen Concentrations by Terminal Electron Accepting Process

Terminal Electron Accepting Process	Typical Hydrogen Concentration (nanomoles/L)
Denitrification	< 0.1
Iron (III) reduction	0.2 to 0.8
Sulfate reduction	1 to 4
Reductive dechlorination	> 1
Methanogenesis	5 to 20

Source: EPA 1998

Redox potential is commonly used to determine whether the subsurface environment is more reducing or more oxidizing. In addition, redox potential can be used to determine the approximate size and shape of a reducing zone at a site. For example, at *Avco Lycoming*, redox potential was used to identify the size and shape of the reducing zones within the plume. Before operation of the system, redox potential was used to show that there were only two relatively small reducing zones, located near the edges of the plume. After 18 months of operation of the system, data on redox potential were used to show that a relatively large reducing zone covered the majority of the plume.

Biological Parameters - In addition to chemical parameters, there are several biological parameters that affect the type of degradation mechanisms that are likely to occur and the rate of degradation. For *in situ* bioremediation of CAHs, common biological parameters include the presence of specific and non-specific microbes and microbial activity. The presence and concentration of non-specific microbes, generally measured as total organic carbon, is used to estimate the abundance of microbes at a site. The presence and concentration of specific microbes is used to determine the concentration of the targeted type of organism at the site. Microbial activity, measured in terms of oxygen uptake rate or dehydrogenate activity, is used to quantify the rate of activity of the targeted microbes.

4.1.2 Site Conditions and Engineered Solutions

In evaluating sites for *in situ* bioremediation, data on hydrogeologic and general aquifer chemistry can be used to identify sites as having generally favorable conditions for *in situ* bioremediation of CAHs. Exhibit 4-4 provides a summary of the generally favorable and unfavorable conditions for *in situ* bioremediation, and typical engineered solutions for unfavorable conditions. As shown in Exhibit 4-4,

examples of generally favorable conditions include highly permeable, homogenous hydrogeology with sufficient nutrients and primary reactants present in the aquifer. Conversely, examples of generally unfavorable conditions include low permeability, highly stratified hydrogeology with insufficient nutrients and primary reactants present in the aquifer.

For sites with unfavorable conditions for *in situ* bioremediation of CAHs, engineered solutions may be available to improve suitability of the hydrogeologic conditions and/or aquifer chemistry of the site for *in situ* bioremediation. Examples of engineered solutions, shown in Exhibit 4-4, include hydrofracturing to increase permeability, and the addition of nutrients and reactants to promote biodegradation. It is important to note that an engineered solution may not be available to modify every unfavorable site condition (identified as “none typically employed” in Exhibit 4-4). In addition, the conditions in Exhibit 4-4 are intended to provide an overview of the types of site conditions that are generally favorable or unfavorable to the use of *in situ* bioremediation. As with any technology, the site-specific conditions should be fully evaluated, along with potential engineered solutions, in determining the applicability of *in situ* bioremediation.

Exhibit 4-4: Generally Favorable and Unfavorable Site Conditions for *In Situ* Bioremediation of CAHs and Typical Engineered Solutions

Conditions		Typical Engineered Solution for Unfavorable Conditions
Favorable	Unfavorable	
Hydrogeologic		
Granular porous media	Fractured rock	None typically employed
High permeability (K > 10 ⁻⁴ cm/s)	Low permeability (K < 10 ⁻⁴ cm/s)	Hydrofracturing and pneumatic fracturing
Saturated media	Unsaturated media	Water application
Minimal heterogeneity	Highly stratified deposits	None typically employed
Aquifer Chemistry		
Minimal NAPL in target area	Significant NAPL in target area	Source containment, treatment, or removal
pH between 6 and 8	pH extremes	Chemical additives (NaHCO ₃ as a buffer)
Nontoxic contaminant concentrations	Toxic contaminant concentrations	Dilution by injection of water or bioremediation additives
Simple contaminant mixtures	Complex contaminant mixtures	None typically employed
Moderate to high microbial activity of appropriate microbes	Little microbial activity or inappropriate microbes	Bioaugmentation
Sufficient nutrients present	Insufficient nutrients present	Addition of nutrients
Sufficient primary reactants	Insufficient primary reactants	Add reactants needed to employ specific mechanism (refer to Exhibit 4-5)

Sources: RTDF 1997; Munakata-Marr and others 1997; ITRC 1998; USAF 1998

4.1.3 Primary Reactants and Additives Typically Employed

Exhibit 4-5 summarizes the primary reactants and additives that are typically employed for engineered *in situ* bioremediation systems. As shown in the exhibit, the types of reactants and additives vary by specific engineered bioremediation mechanism (direct aerobic oxidation, cometabolic aerobic oxidation, and anaerobic reductive dechlorination), and by the targeted CAHs.

For direct aerobic oxidation, primary reactants include oxygen and CAH and possible additives include air, oxygen, hydrogen peroxide (H₂O₂) and magnesium peroxide. For cometabolic aerobic oxidation, additional primary reactants include organic or anthropogenic carbon and additional additives include methane, propane, butane, and ammonia. For anaerobic reductive dechlorination, primary reactants include hydrogen, organic carbon, carbon from a contaminant source (anthropogenic), and CAH while possible additives include lactate, methanol, hydrogen, and molasses. The additives listed in Exhibit 4-5 could be used in any of the configurations described in Section 3, as determined by the specific requirements of the site.

Exhibit 4-5: Primary Reactants and Additives Typically Employed for Engineered *In Situ* Bioremediation of CAHs

Engineered Bioremediation Mechanism	Targeted CAHs	Typical Primary Reactants and Additives in Engineered Systems	
		Primary Reactants	Typical Additives (primary reactant supplemented)
Aerobic oxidation (direct)	DCE, VC, DCA, CA, MC, CM	Oxygen, CAH	Air, oxygen, hydrogen peroxide, magnesium peroxide (oxygen)
Aerobic oxidation (cometabolic)	TCE, DCE, VC, TCA, CF, MC	Oxygen	Air, oxygen, hydrogen peroxide, magnesium peroxide (oxygen)
		Organic carbon or carbon from a contaminant source (anthropogenic)	Methane, propane, butane, ammonia (organic carbon)
Anaerobic reductive dechlorination	PCE, TCE, DCE, VC, TCA, DCA, CT, CF, MC	Hydrogen, organic carbon, or carbon from a contaminant source (anthropogenic)	Lactate, methanol, hydrogen, molasses (electron donor)

Sources: Anderson and Lovley 1997; McCarty 1994; McCarty and others 1998; USAF 1998; EPA 1998; Yager and others 1997

4.1.4 Treatability (Bench-Scale) Testing

Treatability (bench- or laboratory-scale) testing is generally conducted after site characteristics, degradation mechanisms, and potential enhancements have been identified. Treatability testing is used to evaluate the effectiveness of degradation mechanisms and enhancements that are being considered for the site. For example, results of treatability testing can be used to determine the conditions under which degradation products are produced, the rates of degradation, and the paths of degradation in order to identify the specific formulation that supports the most complete and rapid biodegradation of the targeted CAHs. Examples of treatability tests for *in situ* bioremediation of CAHs include microcosm bottle studies and soil column studies. Typically, samples of the media to be treated at the site (groundwater, sediment, or soils) are used in the treatability tests. Because microbial populations usually are heterogeneous in the subsurface and the type of plume may vary across the site, treatability tests are often conducted using samples from several areas of a site.

In addition, data from treatability testing can help identify the parameters to be used for field-scale testing and implementation. It should be noted that rates of biodegradation observed during bench-scale microcosm studies typically will be higher than those observed in the field and that *in situ* residence times will require adjustment accordingly (U.S. Air Force 1998).

The specifics of treatability testing for *in situ* bioremediation can be complex, and are influenced by site-specific conditions. Additional details on designing and conducting treatability tests for *in situ* bioremediation can be found in the references listed in Sections 5 and 6 of this report.

4.1.5 System Design, Field Testing, and Implementation

Information from the first four steps in the selection and implementation of *in situ* bioremediation (shown in Exhibit 4-1) is used in designing the system, testing the system in the field, and implementing the technology on a full-scale basis. As discussed above, treatability testing is generally used to determine the specific formulation (combination of substrates or nutrients as amendments) that supports the most complete and rapid biodegradation of the targeted CAHs. The technology is then scaled-up for field or pilot-scale testing, and configured on a full-scale basis for the conditions specific to the site.

System Design - In the system design stage, a system configuration such as groundwater recirculation or direct injection (see Section 3) that is appropriate for the site conditions and remedial goals is paired with one or more enhancement technologies. Major considerations for system design include the type and size of extraction and injection systems, the arrangement of plumbing and other infrastructure, the method and schedule for addition of amendments, monitoring system equipment, and monitoring schedule. Additional considerations include design of above-ground components, such as storage containers, pumps, mixers, and flow meters. (ITRC, 1998)

In general, for a groundwater recirculation system, well spacing is designed to be at a distance sufficient to observe measurable changes in contaminant concentrations between two or more wells. Residence time or period is the amount of time required for a pollutant molecule to pass through the contaminated area, and is measured with a variety of techniques. For example, bromide tracer studies are used to determine actual travel time between the injection and the extraction wells.

Pilot/Field-Scale Testing - Pilot- or field-scale testing involves installation of an extraction and/or injection system at a site, and operation of that system over time. While pilot/field-scale systems vary in size, they are usually constructed on a smaller scale than a full-scale system.

For example, a small pilot-scale test of a groundwater recirculation system can be designed using the ESTCP technical protocol. Their design includes a system with three 2-inch injection wells spaced along 12-inch centers, one 2-inch extraction well down-gradient from the injection wells, and one 4-inch extraction well further down-gradient. This design indicates that the system be oriented parallel to the natural flow direction, and that the spacing between injection and extraction wells be the distance that groundwater would travel in 35 - 40 days under natural flow conditions. The design also includes three rows of monitoring wells between the injection and extraction wells. (ESTCP, 1998)

An important aspect of pilot/field-scale testing is the evaluation of the results from testing to determine system effectiveness. AFCEE has suggested three lines of evidence for performance evaluation:

1. Reduction in contaminant mass - this includes temporal and spatial reductions in concentrations; integration of extrapolated concentration measurements for the system; comparison of concentrations through multiple recirculation cycles and addition of amendments; and comparison of mass leaving injection points and arriving at extraction points
2. Microbiological activity linked to degradation - this includes microcosm or column studies which demonstrate metabolic activity; demonstrations showing that calculated field

degradation rates are consistent with microcosm or column studies; and correlation of biomass in the field with zones of contaminant depletion

3. Contaminant disappearance linked to cometabolic system (only for aerobic cometabolic systems) - correlation of contaminant depletion with substrate depletion; correlation of temporal changes in contaminant concentration with addition of substrate; and evidence showing aerobic conditions, such as high oxidation reduction potential

To evaluate pilot/field test results, amended groundwater can be sampled and the results compared with up-gradient contaminated conditions as it passes each monitoring point, or once steady-state conditions have been achieved and for several residence periods. Frequently, control plots with unamended groundwater are sampled to provide a basis of comparison with the amended groundwater plots.

When multiple formulations are being pilot-tested, parallel plots can be used to allow simultaneous testing of alternative formulations. Parallel plots also can be used to determine the lateral extent of biodegradation, especially from monitoring locations perpendicular to groundwater flow near a test plot, also referred to as **diffusion** degradation.

For some *in situ* bioremediation applications, site remedial goals might be achieved after the conclusion of field testing. In other cases, the pilot system is scaled up to a full-scale system to remediate the site. Because of the variability of subsurface conditions, field testing of an *in situ* bioremediation process may be iterative, requiring several preliminary designs and field tests to determine the best configuration for a full-scale system. It is also possible that an enhancement technology shown to be effective in the laboratory is ineffective in the field, possibly requiring additional site characterization and re-evaluation of appropriate *in situ* bioremediation enhancement technologies.

Example of Field Testing and Implementation - Field testing and implementation for *in situ* bioremediation systems for cleanup of **CAHs** is illustrated in the following example, as shown in the case studies discussed in Appendix A of this report.

At the Dover Air Force Base Building 719 site, a pilot test that used cometabolic bioventing was conducted over 14 months. Before the pilot test began, laboratory tests were performed on soils from the area of Building 719 to evaluate candidate substrates. Propane was identified as the preferred substrate to be tested in the pilot study because of its ability to stimulate cometabolic activity affecting both TCA and TCE, the two contaminants of interest at the site.

Full-Scale Implementation - Once pilot/field testing is complete, the next step in the process is the design (scale-up) of the full-scale system. As discussed earlier, the full-scale system is configured according to the conditions specific to the site. Other considerations in designing the full-scale system include aerial extent and depth of contamination to be remediated, types and concentrations of contaminants and the remedial goals, other regulatory considerations, timeframe for remediation to be completed, and cost.

The first step in a full-scale application is to perform system start-up, where the activity of the microbial populations are acclimatized to the added amendments. After start-up, routine operation and maintenance is generally performed, including monitoring of system performance. Many of the same types of data gathered and evaluated during a pilot/field-scale test are collected for full-scale applications, including information about reductions in contaminant mass and microbiological activity linked to degradation.

Clogging of injection and extraction wells is a particularly important consideration during full-scale implementation of an *in situ* bioremediation system. Clogging occurs when there is a buildup of biomass on or near the well screens, making it difficult to inject or extract fluids from the subsurface. Techniques that have been used to reduce the impacts from clogging include pulsed addition of substrates, use of reduced concentrations of substrates, and routine well cleaning. Pulsed addition of substrates (for a cometabolic system) has the added advantage of providing limited slugs of substrate that can be periodically depleted, thus reducing the competitive inhibition of the primary substrate on the targeted chlorinated compound. (ITRC, 1998)

Additional information about full-scale design and implementation of *in situ* bioremediation can be found in sections 5 and 6 of this report. Example resources include the AFCEE's guidance manual and screening software for *in situ* bioremediation and ITRC's document about technical and regulatory requirements for enhanced *in situ* bioremediation, described briefly below.

- Aerobic Cometabolic *In Situ* Bioremediation Technology Guidance Manual and Screening Software User's Guide; AFCEE; June 1998 (available on the Internet at <http://en.afit.af.mil/env/insitubio.htm>). This guide presents the principles of aerobic cometabolic *in situ* bioremediation, the mathematical models used to describe the technology, and a discussion of the applicability and limitations of the technology. In addition, a software program that may be used to help determine whether the technology is appropriate for implementation at a given site is available at the Internet site listed above. The steps needed to design and implement the technology, a discussion of regulatory acceptance, and case studies of use of the technology also are presented.
- Technical and Regulatory Requirements for Enhanced *In Situ* Bioremediation of Chlorinated Solvents in Groundwater; ITRC; *In Situ* Bioremediation Subgroup; December 23, 1998 (available on the Internet at <http://itrcweb.org/reports/isb.htm>). This document provides technical and regulatory information that state and federal regulators need to support decisions about whether to proceed with field studies of enhanced *in situ* bioremediation, and to implement such pilot tests successfully.

Example of Full-Scale Implementation - Full-scale implementation is illustrated in the following example, as shown in the case studies in Appendix A of this report.

At the abandoned manufacturing facility site in Emeryville, California, a pilot study was conducted for approximately six months to determine whether the rate of anaerobic dechlorination could be enhanced by the addition of a molasses solution to the groundwater. The pilot study demonstrated that the rate was enhanced through addition of the molasses, and the technology subsequently was used on a full-scale basis. Groundwater monitoring data collected after the system had operated for 18 months showed that the pilot-scale system had been a good indicator of the performance that the system could achieve.

4.2 ADDITIONAL SELECTION FACTORS

Additional factors relevant to the selection of *in situ* bioremediation as a treatment for CAHs include (ITRC 1998):

- Advantages and potential limitations of the technology
- Regulatory considerations

Advantages and Potential Limitations of the Technology

In situ bioremediation is used at sites at which soil or groundwater is contaminated with CAHs. Advantages of using the technology include:

- Capability to degrade CAH contaminants to relatively less toxic end products
- Generation of relatively small amounts of remediation wastes, compared to *ex situ* technologies
- Reduced potential for cross-media transfer of contaminants commonly associated with *ex situ* treatment
- Reduced risk of human exposure to contaminated media, compared to *ex situ* technologies
- Relatively lower cost of treatment compared to excavation and disposal, *ex situ* treatment, or conventional pump-and-treat systems
- Potential to remediate a site faster than with use of conventional technologies (EPA 1999)

Potential limitations of using *in situ* bioremediation include:

- A perceived lack of knowledge about biodegradation mechanisms
- Specific contaminants at a site may not be amenable to biodegradation
- Enhancement technologies, when needed, may be costly or their implementation may be technologically challenging
- The toxicity of transformation (daughter) products may exceed that of parent compounds

Regulatory Considerations

As with any technology, there are a number of regulatory considerations that may impact the selection and implementation of *in situ* bioremediation as a remedy for a site. The following presents general information about some of the regulations that are relevant to *in situ* bioremediation. It is important to note that the determination of what regulations (federal, state, and local) apply to a specific technology application must be made on a case-by-case basis.

Because *in situ* bioremediation typically involves the direct injection of chemicals into the subsurface or the pumping and reinjecting of “activated” groundwater, the Underground Injection Control (UIC) regulatory program under the Safe Drinking Water Act is often a consideration. UIC regulations prohibit the injection of fluid that contains a contaminant into an underground source of drinking water if the presence of the contaminant may cause a violation of any primary drinking water regulation under 40 Code of Federal Regulations (CFR) Part 142 or otherwise have an adverse effect on the health of persons. Under 40 CFR 144.11, any underground injection, except into a well authorized by rule or except as authorized by permit issued under the UIC program, is prohibited. Under 40 CFR 144.12(a), no owner or operator shall construct, operate, maintain, convert, plug, abandon, or conduct any other injection activity in a manner that allows the movement of fluid containing any contaminant into underground sources of drinking water, if the presence of that contaminant may cause a violation of any primary drinking water regulation under 40 CFR 142 or may otherwise adversely affect the health of

persons. UIC regulations define five “classes” of injection wells (see 40 CFR 144.6). Under the federal UIC regulations, the injection of hazardous waste (as might be the case in a groundwater recirculation system) into a formation that is within or above an underground source of drinking water (deemed a Class IV well) generally is prohibited, unless the injection is part of an approved RCRA corrective action or CERCLA response (see 40 CFR 144.13). In such cases, it may be necessary to use other means of groundwater reinjection (such as recharge trenches). In cases in which the injected material is not a hazardous waste or in which hazardous waste is being injected into an aquifer that is not an underground source of drinking water, a Class V injection well permit may be required³. In addition, establishment of a “containment area” is sometimes required to demonstrate that injected products are not migrating from the site.

Contaminated soil or groundwater may be identified as a RCRA hazardous waste either because it is a listed hazardous waste (such as F001 through F005, spent solvents) or because it exhibits a characteristic of hazardous waste (such as the Toxicity Characteristic [TC]). Under RCRA, the injection of a hazardous waste into an aquifer is defined as “land disposal”, and therefore is subject to the RCRA Land Disposal Restriction (LDR) regulations under 40 CFR Part 268. These restrictions may prohibit the injection of hazardous wastes into an aquifer until the groundwater is treated (1) to specified concentrations, or (2) using a prescribed technology. However, section 3020(b) of RCRA provides a statutory waiver for compliance with LDRs that may permit reinjection of contaminated groundwater that is a hazardous waste. In order to qualify for this waiver, the reinjection process must meet all of the following criteria:

- Must be performed under CERCLA response or RCRA corrective action authorities.
- Can occur only after the groundwater has been treated to “substantially reduce hazardous constituents” prior to reinjection.
- Must be part of a cleanup process that will, upon completion, be sufficient to protect human health and the environment.

RCRA section 3020(a) bans hazardous waste disposal by underground injection into or above an underground source of drinking water (within one-quarter mile of the well). However, as discussed above, section 3020 (b) exempts from the ban the reinjection of contaminated groundwater if certain conditions are met. To facilitate the use of *in situ* bioremediation of contaminated groundwater, EPA has clarified the applicability of RCRA section 3020(b) to *in situ* bioremediation technologies. In a December 1999 letter to an official in the California hazardous waste program, titled *Applicability of RCRA 3020(B) to In Situ Bioremediation Technologies* (available at <http://clu-in.org>), EPA stated that the addition of nutrients or other products designed to promote *in situ* bioremediation is considered “treatment” under section 3020(b), and that the substantial reduction of hazardous constituents required may occur before or after reinjection. This clarification allows for *in situ* bioremediation of groundwater without an above-ground pump-and-treat system in place, as long as the other conditions of section 3020(b) discussed above are met.

³ Permits are not required for on-site CERCLA response actions. However, the substantive requirements of permits (that is, closure requirements governing closure of injection wells) must be met for CERCLA actions.

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APPENDIX A

CASE STUDIES AND SUMMARIES OF SITES USING *IN SITU* BIOREMEDIATION TECHNOLOGIES FOR CAHs

Appendix A includes nine case studies prepared by EPA of sites which have used, or are in the process of using, *in situ* bioremediation for treatment of CAHs in contaminated soil and groundwater. In addition, summary information about other full-scale and pilot-scale applications of *in situ* bioremediation based on information from the proceedings of the Fifth International and On-Site Bioremediation Symposium and from the Bioremediation the Field Search System (BFSS) is presented.

The nine case studies, summarized in Exhibit A-1, contain information about site history and source of contamination, geology/hydrogeology/contaminant characterization, technology description, technology performance and cost, summary observations and lessons learned, contact information, and references used in the preparation of the case studies. The case studies include three full-scale applications of anaerobic reductive dechlorination (*Texas Gulf Coast*, *Avco Lycoming*, and *Emeryville*) and two field demonstrations of anaerobic reductive dechlorination (*Watertown* and *Dover Area 6*). Four case studies address field demonstrations of aerobic oxidation (*Moffett Field*, *Edwards AFB*, and *SRS* for groundwater and *Dover Building 719* for soil).

To provide additional information about full-and pilot-scale *in situ* bioremediation applications, summary information was obtained from the proceedings of the Fifth International *In Situ* and On-Site Bioremediation Symposium and from the BFSS. While not comprehensive, the summary information presented in Exhibits A-2 and A-3 provides an overview of the types of sites using *in situ* bioremediation.

- Exhibit A-2 presents a summary of information about 8 full-scale applications and 14 pilot studies that are described in the proceedings of the Fifth International *In Situ* and On-Site Bioremediation Symposium. This exhibit presents the site name and location, technology, media, contaminants, period of performance, and points of contact for each of the *in situ* bioremediation full-scale applications and pilot studies identified in the proceedings. Most of the projects involved treatment of chlorinated solvents by either anaerobic reductive dechlorination, aerobic oxidation, or a combination of those two technologies.
- Exhibit A-3 provides summary information about remediation efforts at 8 sites that are described in the BFSS, including site name and location, technology, media, contaminants, project status, and points of contact for the applications.

Exhibit A-1: Summary of Case Studies of *In Situ* Bioremediation Technology Applications

#	Site Name, Location	Mechanism(s)	Technology/Configuration	Technology Scale	Matrix Treated	Contaminants Targeted ¹	Period of Operation
1	NAS Moffett Field, Mountain View, California	Aerobic oxidation (cometabolic and direct)	Electron acceptor (EA) addition (oxygen and hydrogen peroxide) Electron donor (ED) addition (methane, toluene, and phenol) <i>Groundwater recirculation</i>	Field demonstration	Groundwater	TCE, cis-DCE, trans-DCE, VC	9/86-11/88
2	Edwards Air Force Base (AFB), California	Aerobic oxidation (cometabolic and direct)	EA addition (oxygen and hydrogen peroxide) ED addition (toluene) <i>Groundwater recirculation</i>	Field demonstration	Groundwater	TCE	2/5/96-4/1/97
3	U.S. Department of Energy Savannah River Site, Aiken, South Carolina*	Aerobic oxidation (cometabolic and direct)	Nutrient addition EA addition (oxygen) ED addition (methane) <i>Direct injection</i>	Field demonstration	Sediment and Groundwater	TCE, PCE	2/26/92-4/30/93
4	Texas Gulf Coast Site (site name confidential) Houston, Texas**	Anaerobic reductive dechlorination (cometabolic and direct)	Nutrient addition ED addition (methanol) <i>Groundwater recirculation</i>	Full	Groundwater	TCE, cis-1,2-DCE, VC	Ongoing (data available from 6/95 - 12/98)
5	Avco Lycoming Superfund Site Williamsport, Pennsylvania	Anaerobic reductive dechlorination (cometabolic and direct)	ED addition (molasses) <i>Direct injection</i>	Pilot and full	Groundwater	TCE, DCE, VC, Cr ⁺⁶ , Cd	Pilot study 10/95-3/96; Full scale Ongoing (data available from 1/97 - 10/97)
6	Abandoned Manufacturing Facility Emeryville, California	Anaerobic reductive dechlorination (cometabolic and direct)	ED addition (molasses) <i>Direct injection</i>	Pilot and full	Groundwater	TCE, Cr ⁺⁶	Pilot study 8/95-2/96; Full scale Ongoing (data available from 4/97 - 10/98)

Exhibit A-1: Summary of Case Studies of *In Situ* Bioremediation Technology Applications (continued)

#	Site Name, Location	Mechanism(s)	Technology/Configuration	Technology Scale	Matrix Treated	Contaminants Targeted ¹	Period of Operation
7	Watertown, Massachusetts**	Anaerobic reductive dechlorination (cometabolic and direct) Aerobic oxidation (cometabolic and direct)	Nutrient addition ED addition (lactate) <i>Groundwater recirculation</i> EA addition (oxygen) ED addition (propane) <i>Groundwater recirculation</i>	Field demonstration (SITE test)	Groundwater	PCE, TCE	Anaerobic, 11/96-7/97; Aerobic, ongoing (data available from 8/97 - 10/97)
8	Dover AFB Area 6 Dover, Delaware	Anaerobic reductive dechlorination (cometabolic and direct)	Bioaugmentation Nutrient addition ED addition (lactate) <i>Groundwater recirculation</i>	Field demonstration (proof of technology test)	Groundwater	TCE	9/96 - 3/98
9	Dover AFB Building 719 Dover, Delaware	Aerobic oxidation (cometabolic and direct)	EA addition (oxygen) ED addition (propane) <i>Direct injection</i>	Field demonstration (pilot test)	Soil	TCE, TCA, DCE	5/98 - 7/99

* Remediation efforts at this site also are included in Exhibit A-2 (Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings of the Fifth International *In Situ* and On-Site Bioremediation Symposium) and Exhibit A-3 (Summary of Full-scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System [BFSS]).

** Remediation efforts at these sites also are included in Exhibit A-2 (Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings of the Fifth International *In Situ* and On-Site Bioremediation Symposium).

¹ Contaminant Key: Cd = Cadmium, Cr = Chromium, DCE = dichloroethene, PCE = tetrachloroethene, TCA = trichloroethane, TCE = trichloroethene, VC = vinyl chloride

Exhibit A-2: Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings of The Fifth International *In Situ* and On-Site Bioremediation Symposium

Site Name, Location	Technology	Media	Contaminants ¹	Period of Performance	Points of Contact	Page No. in Proceedings
FULL-SCALE APPLICATIONS						
Site name not provided (commercial laundry) southwestern Ohio	Aerobic oxidation; injection of propane	Groundwater	PCE, TCE, 1,2-DCE	June 1998 - pilot test September 1998 - begin full scale; end date not provided	Peter I. Dacyk William D. Hughes Parsons Engineering Science, Inc. 13 Triangle Park Drive, Suite 1302 Cincinnati, Ohio 45246	1
Site name not provided (aluminum coating facility) location not provided (midwestern state)	Aerobic oxidation (oxygen release compound [ORC] slurry injection)	Groundwater	VC	March 1998 - injection	Peter L. Tacy, Jr. STS Consultants, Ltd. 1502 Randolph Street, Suite 100 Detroit, Michigan 48226-2216	15
Site name not provided (dry cleaning site) Wisconsin	Anaerobic reductive dechlorination (injection of glycerol polylactate hydrogen release compound [HRC])	Groundwater	PCE	Not provided	John K. Sheldon Montgomery Watson 11153 Aurora Avenue Des Moines, Iowa 50322-7238 Kenneth J. Quinn, Montgomery Watson, Madison, Wisconsin Stephen S. Koenigsberg and Craig A. Sandefur, Regenesys, San Juan Capistrano, California	61
Site name not provided (natural gas pipeline compressor station) Virginia	Aerobic oxidation (Methanotrophic Treatment Technology [MTT], injection of methane, air, nitrous oxide, and triethylphosphate)	Groundwater	PCE, TCE	March 1998 - ongoing (end date not provided)	Mark S. Nelson, Williams Gas Pipeline-Transco, Houston, Texas Robert Legrand and Andrew J. Morecraft Radian International 8501 N. Mopac Blvd P.O. Box 201088 Austin, Texas 78720-1088 (and Raleigh-Durham, North Carolina) John A. Harju, Gas Research Institute, Chicago, Illinois	113

Exhibit A-2: Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings from The Fifth International *In Situ* and On-Site Bioremediation Symposium (continued)

Site Name, Location	Technology	Media	Contaminants ¹	Period of Performance	Points of Contact	Page No. in Proceedings
FULL-SCALE APPLICATIONS (continued)						
Site name not provided Watertown, Massachusetts	Anaerobic reductive dechlorination (addition of HRC to a recirculation cell)	Groundwater	PCE, TCE, DCE, VC	February 1998 - January 1999	Maureen A. Dooley and Willard A. Murray, Harding Lawson Associates 107 Audubon Road, Suite 300 Wakefield, Massachusetts 01880 Stephen Koenigsberg, Regensis, San Juan Capistrano, California	121 **
Site name not provided (manufacturing facility) Texas Gulf Coast, Texas	Anaerobic reductive dechlorination (addition of methanol to recirculation system)	Groundwater	TCE	June 1995 - December 1998	Susan Tighe Litherland, P.E. and David W. Anderson Roy F. Weston, Inc. Building 1, Suite 100 5300 Bee Caves Road Austin, Texas 78746-5225 Blake A. Dinwiddie, Roy F. Weston, Inc., Houston, Texas	157 **
Site name not provided Lansing, Michigan	Aerobic oxidation (cometabolic) (phenol addition with pressurized fluidized bed reactor); Groundwater recirculation; Biosparging	Groundwater	TCE	May 1997 - end date not provided (data available for 15 months of operation)	Jian Xing and Richard M. Raetz Global Remediation Technologies, Inc. 1235 Woodmere Traverse City, MI 49686	217
Evenblij site, Hoogeveen, The Netherlands	Aerobic oxidation (acetate and lactate addition); Anaerobic reductive dechlorination; Groundwater recirculation	Groundwater	PCE, TCE	Not provided	M.J.C. Henssen Bioclear Environmental Biotechnology Groningen, The Netherlands C. Hubach and R. Blokzijl DHV Environmental & Infrastructure Europaweg 33/2 9723 AS Groningen, The Netherlands J. Mourik, Logisticon Water Treatment, Groot-Amers, The Netherlands E. Meijerink, Province of Drenthe, Assen, The Netherlands	225

Exhibit A-2: Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings from The Fifth International *In Situ* and On-Site Bioremediation Symposium (continued)

Site Name, Location	Technology	Media	Contaminants ¹	Period of Performance	Points of Contact	Page No. in Proceedings
PILOT STUDIES						
Hill Air Force Base, Operable Unit 1, Chemical Disposal Pit 1 Ogden Utah	Bioventing	Soil	1,2-Dichlorobenzene	One year (dates not provided)	James T. Gibbs, Battelle 505 King Avenue Columbus, Ohio 43201 R. Kenneth Crowe, TRW Inc., Tyndall AFB, Florida Jon Ginn, USAF, Hill AFB, Utah	7
Site name not provided Copenhagen, Denmark	Aerobic oxidation; injection of methane and air	Groundwater	cis-1,2-DCE, VC	July 1998 - ongoing; end date not provided	Liselotte Ludvigsen HOH Water Technology, Greve, Denmark Kim Broholm, VKI Agern Alle 11 DK-2970 Horsholm, Denmark Lars Deigaard, Scanrail Consult, Copenhagen, Denmark	81
U.S. Department of Energy Savannah River Site, Sanitary Landfill Aiken, South Carolina	Aerobic oxidation; injection of methane, air, triethyl phosphate, and nitrous oxide	Groundwater	TCE, cis-1,2-DCE, VC	September 1996	Robin L. Brigmon Westinghouse Savannah River Company Savannah River Technology Center P.O. Box 616 Aiken, South Carolina 29808 Terry C. Hazen, Lawrence Berkeley National Laboratory, Berkeley, California Al W. Bourquin, Camp Dresser and Mckee Inc., Denver, Colorado	107 **
Site name not provided (Superfund site) Location not provided	Anaerobic reductive dechlorination	Groundwater	PCE, TCE, cis-1,2-DCE, VC	April 1997 - August 1998	James J. Reid, P.E. and Denis Balcer ARCADIS Geraghty & Miller 4700 Lakehurst Court, Suite 100 Dublin, Ohio 43016	135

Exhibit A-2: Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings from The Fifth International *In Situ* and On-Site Bioremediation Symposium (continued)

Site Name, Location	Technology	Media	Contaminants ¹	Period of Performance	Points of Contact	Page No. in Proceedings
PILOT STUDIES (continued)						
Site name not provided (industrial cleaning company) Arnhem, The Netherlands	Anaerobic reductive dechlorination	Soil	PCE, TCE, cis-1,2-DCE, VC	80 days (dates not provided)	H. Slenders TNO Institute of Environmental Sciences, Energy Research and Process Innovation Business Park E.T.V. Laan van Westenenk 501 P.O. Box 342 7300 AH Apeldoorn, The Netherlands S. Hofstra, IWACO, Environmental Consultants, The Netherlands H.de Sain, BdS, Management Consultancy, The Netherlands R. Hetterschijt, Netherlands Institute of Applied Geoscience TNO H. de Kreuk, BioSoil R&D	141
Idaho National Engineering and Environmental Laboratory, Test Area North Idaho	Anaerobic reductive dechlorination	Groundwater	TCE	November 1998 - ongoing; end date not provided	Kent S. Sorenson, Jr. and Lance N. Peterson Lockheed Martin Idaho Idaho Falls, Idaho Roger L. Ely, University of Idaho, Moscow, Idaho	147
Naval Air Station Point Mugu, IRP Site 24, California	Anaerobic reductive dechlorination (Phase 1 – lactate addition; Phase 2 – nutrient injection); groundwater recirculation	Groundwater	Chlorinated ethenes; volatile organic acids	Not provided	Christian D. Johnson Battelle PNWD P.O. Box 999 Richland, Washington 99352 Daniel P. Leigh and Lisa A. Bienkowski, IT Group, Pleasanton, California Steve Granade, U.S. Navy, Naval Construction Battalion Center, Port Hueneme, California Bryan Harre, U.S. Navy, Port Hueneme, California Todd Margrave, U.S. Navy, San Diego, California	165

Exhibit A-2: Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings from The Fifth International *In Situ* and On-Site Bioremediation Symposium (continued)

Site Name, Location	Technology	Media	Contaminants ¹	Period of Performance	Points of Contact	Page No. in Proceedings
PILOT STUDIES (continued)						
Site name not provided (former wastewater treatment facility) Location not provided	Anaerobic reductive dechlorination (yeast extract injection)	Groundwater	PCE, TCE, 1,1,1-TCA, 1,1-DCE, cis/trans-1,2-DCE, 1,1-DCA, 1,2-DCA, VC	July 1997 – November 1998	I. Richard Schaffner, Jr., GZA GeoEnvironmental, Inc. 380 Harvey Road Manchester, New Hampshire 03103	171
Site name not provided Florida	Anaerobic reductive dechlorination (addition of HRC)	Groundwater	TCE, cis-1,2-DCE, VC	March 1998 – June 1998	Madeline Wu Water Restoration Inc. Fort Lauderdale, Florida	177
Site name not provided New Jersey	Anaerobic reductive dechlorination (addition of HRC)	Groundwater	PCE, TCE, cis-1,2-DCE	April 1998 – June 1998	S. Kallur, Environmental Strategies & Applications, Inc. Somerset, New Jersey S. Koenigsberg, Regenesis 1011 Calle Sombra San Clemente, CA 92672	181
Offutt Air Force Base, Fire Protection Training Area 3 Nebraska	Anaerobic reductive dechlorination (direct addition of hydrogen); groundwater recirculation	Groundwater	cis-1,2-DCE, BTEX	November 1998	R. Todd Fisher and Charles J. Newell , Groundwater Services, Inc., Houston, Texas Patrick E. Haas, Air Force Center for Environmental Excellence, Brooks AFB, Texas Joseph B. Hughes, Rice University, Houston, Texas	185
Site name not provided (industrial site) Adelaide, South Australia	Chemical oxidation, with addition of permanganate	Groundwater	TCE	Not provided	Christopher H. Nelson, IT Corporation, Englewood, Colorado Craig S. Barker, IT Environmental (Australia) Pty Ltd, Adelaide, South Australia	199

Exhibit A-2: Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings from The Fifth International *In Situ* and On-Site Bioremediation Symposium (continued)

Site Name, Location	Technology	Media	Contaminants ¹	Period of Performance	Points of Contact	Page No. in Proceedings
PILOT STUDIES (continued)						
ITT Industries Night Vision Roanoke, Virginia	Aerobic oxidation (injection of methane, air, nitrous oxide, and triethylphosphate)	Groundwater	TCE, TCA, acetone, isopropanol	Not provided	Gregory L. Carter, Earth Tech 5320 Peters Creek Road, Suite D Roanoke, Virginia 24019 Jennifer C. Vincent and Barbara B. Lemos, Earth Tech, Concord, Massachusetts Rosann Kryczkowski, ITT Industries Night Vision, Roanoke, Virginia	255
Rickenbacker Air National Guard Base Ohio	Lasagna™ (combination of aerobic cometabolic-bioremediation [with addition of methane], electroosmosis, and hydraulic fracturing)	Soil	TCE	January 1997 - November 1998	Wendy J. Davis-Hoover et. al., EPA CHL, EPA Facilities 26 W. Martin Luther King Drive Cincinnati, Ohio 45268 Taras Bryndzia, Shell Oil, Houston, Texas Michael H. Roulier and Lawrence C. Murdoch, Clemson University, Clemson, South Carolina Mark Kemper and Philip Cluxton, Cluxton Instruments, Inc., Martinsville, Ohio Souhail Al-Abed and William Slack, FRX, Inc., Cincinnati, Ohio	263

* These full-scale applications and pilot studies are described further in the following document: Andrea Leeson, A. and B. C. Alleman. *Engineered Approaches for In Situ Bioremediation of Chlorinated Solvent Contamination 5(2): The Fifth International In Situ and On-Site Bioremediation Symposium; San Diego, California, April 19-22, 1999*; Battelle Press; 1999. Page numbers shown in Exhibit A-2 refer to the pages of the Battelle document in which the information can be found.

** These full-scale applications and pilot studies are also included in Exhibit A-1: Summary of Case Studies of *In Situ* Bioremediation Technology Applications.

*** This pilot study is also included in Exhibit A-3: Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System (BFSS)

¹ Contaminant Key: DCA = dichloroethane, DCE = dichloroethene, PCE = tetrachloroethene, TCA = trichloroethane, TCE = trichloroethene, VC = vinyl chloride

Exhibit A-3: Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System (BFSS)

Site Name, Location	Technology	Media	Contaminants ¹	Project Status	Points of Contact
A. B. Dick Niles, Illinois	Inland Environmental Bio-Treatment Process. An anaerobic, in-situ soil bioremediation technology. A proprietary liquid blend (patent applied for) of nutrients, stimulants, and mobilizing agents is injected into the soil and stimulates the natural anaerobic bio-degradation of contaminants to mineralization	Soil	TCE, cis-1,2-DCE, TCA	Full-scale operations were completed on 8/23/95. The remediation was completed ahead of schedule (three months versus one year) and within the budget. All treatment goals were met and the site is now considered to be remediated.	Gregory C. Weeks Inland Environmental, Inc. 3921 Howard St. Skokie, IL 60076 Inland@Inland-Env.com (847) 677-7500
Barber Greene North Aurora, Illinois	<i>In situ</i> , aerobic process. Oxygen is introduced into the groundwater through air sparging. Vacuum extraction provides air movement through the soil to enhance groundwater bioremediation (bioventing for vadose soil).	Soil (vadose), groundwater	PCE, 1,1-DCA, 1,1,1-TCA, TCE	Full-scale operations were completed on 7/18/97.	Eric Portz Illinois EPA 1021 N. Grand Ave. East Springfield, IL 62702 epa4204@epa.state.il.us (217) 782-6761
Chevron Chemical Company - Berkeley Heights, New Jersey	Aerobic <i>in situ</i> groundwater and soil bioremediation process. Treatment process involves pumping groundwater, adding nutrients (nitrogen and phosphorus) and amendments (hydrogen peroxide and oxygen) above ground, and reinjecting the groundwater into the soil. The reinjected water will help to remediate the contaminated soils. Groundwater that meets cleanup criteria will be sent to a POTW.	Soil (vadose), soil (saturated), groundwater	The system is designed to treat 1,1,1-TCA, 1,2-DCE, and TCE. Other contaminants include PCBs and heavy metals.	The full-scale system began operating on 1/1/95. Mechanical problems caused by microorganism growth occurred.	Robert Huntoon Chevron Chemical Company P.O. Box 6012 San Ramon, CA 94583 RBHU@ CHEVRON.COM (925) 842-5576 Glenn Savary New Jersey Department of Environmental Protection and Energy (609) 633-1408

**Exhibit A-3: Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System (BFSS)
(continued)**

Site Name, Location	Technology	Media	Contaminants ¹	Project Status	Points of Contact
<p>NAS Fallon Fallon, Nevada</p>	<p>Aerobic bioslurping process. A vacuum is pulled in dewatering wells to promote the rapid aeration of subsurface vadose zone soils by movement of air into soil pores. Since the vacuum (slurper) tubes are situated at the interface of the free fuel and the groundwater, free fuel is removed (vacuum assisted), while simultaneously promoting aerobic biodegradation of fuel in the vadose zone through bioventing.</p>	<p>Soil (vadose), soil (saturated), groundwater</p>	<p>System is designed to treat DCE, PCE, trichloroethylene, and cis-1,2-DCE. Other contaminants include perchloroethene and trichloroethene (in vadose zone) and arsenic and borates (natural contaminants).</p>	<p>The full-scale system is operational.</p>	<p>Ron Hoeppel Naval Facilities Engineering Service Center (805) 982-1655 RHOEPPE@ NFESC.NAVY.MIL</p> <p>David Chesmore State of Nevada Federal Facilities Coordinator (702) 687-5872</p> <p>Doug Bonham NAS Fallon Public Works Department 4755 Pasture Rd. Fallon, NV 89496-5000 (702) 426-2772</p>
<p>Naval Submarine Base Kings Bay, Georgia</p>	<p>Aerobic, <i>in situ</i> groundwater and soil bioremediation process. Bacteria, nutrients, and water were injected periodically into the groundwater. Three sites were contaminated with petroleum hydrocarbons (heating fuel, diesel fuel, and lubricating oil).</p>	<p>Groundwater</p>	<p>The system is designed to treat 1,1-DCA, 1,2-DCA, cis-1,2-DCE, PCE, trans-1,2-DCE, and TCE. BTEX is also present in the groundwater.</p>	<p>The full-scale system has been completed, and no problems have been reported. One of the sites (“generator” site) is operational.</p>	<p>John Garner Naval Submarine Base Kings Bay Kings Bay, GA 31547 jgarner@ subase.kb.navy.mil (912) 673-2001</p> <p>J.A. Jones (912) 673-2001</p> <p>Garland Creech Naval Submarine Base Kings Bay (912) 673-2001</p>

**Exhibit A-3: Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System (BFSS)
(continued)**

Site Name, Location	Technology	Media	Contaminants ¹	Project Status	Points of Contact
Pine Bend Landfill Inner Grove Heights, Minnesota	<i>In situ</i> groundwater bioremediation process. A pilot-scale system that consists of three injection wells currently is being installed at the site. The wells will deliver fructose corn syrup to the groundwater to induce anaerobic conditions and stimulate the anaerobic groundwater microbial consortia to effectively dehalogenate highly chlorinated contaminants in groundwater. Groundwater that retains contaminants may be treated aerobically downgradient through installation of a sparging curtain.	Groundwater	The system is designed to treat TCA, DCA, DCE, and perchloroethylene.	The full-scale system is in the predesign phase. Treatment currently is in the feasibility study stage. The pilot study evaluation is expected to continue for 18 months.	<p>Joe Julik Minnesota Pollution Control Agency 520 Lafayette Rd. St. Paul, MN 55155-4194 joe.julik@pca.state.mn.us (612) 296-8454</p> <p>Neil Wilson Minnesota Pollution Control Agency (612) 296-8596</p>

**Exhibit A-3: Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System (BFSS)
(continued)**

Site Name, Location	Technology	Media	Contaminants ¹	Project Status	Points of Contact
Savannah River Site Aiken, South Carolina **	<p><i>Full-Scale System</i> Biosparging, <i>in situ</i> soil flushing, vacuum extraction. Aerobic process. Indigenous microbes are encouraged to degrade contaminants by injecting air in the aquifer. Air is injected via horizontal wells in the aquifer. Air is vacuum-extracted simultaneously through a parallel horizontal well just above the water table. Off-gas is treated by catalytic oxidation.</p> <p><i>Pilot-Scale System</i> Biosparging, <i>in situ</i> methane, <i>in situ</i> soil flushing, vacuum extraction. Aerobic process. Nutrients are methane, nitrous oxide, gas (triethyl phosphate). Methane in air (1 to 4 percent) is injected through horizontal wells in the aquifer. Air is extracted simultaneously through a parallel horizontal well just above the water table. Off-gas is treated by catalytic oxidation. Methane is added to stimulate indigenous methanotrophs (bacteria) to biodegrade chlorinated solvents through cooxidation.</p>	Groundwater	The system treats PCE and TCE. Copper is also present in the groundwater.	<p>Full-scale operations were completed. No date provided.</p> <p>Pilot-scale operations were completed on 4/1/93. The full-scale system design was completed on 9/30/96.</p>	<p>Ed Wilde Westinghouse Savannah River Company Savanna River Technology Center P.O. Box 616 Aiken, SC 29808 ed.wilde@srs.gov (803) 557-7049</p> <p>James A. Wright DOE (803) 725-5608</p>

**Exhibit A-3: Summary of Full-Scale Applications and Pilot Studies Identified in the Bioremediation in the Field Search System (BFSS)
(continued)**

Site Name, Location	Technology	Media	Contaminants ¹	Project Status	Points of Contact
Technical Products, Inc. Chicago, Illinois	Inland Environmental Bio-Treatment Process. <i>In situ</i> , anaerobic process. The site was excavated to remove tanks and the soil was replaced in deep lifts. Inland Environmental's proprietary bio-stimulating solution (nutrients, biostimulants, and surfactants) was sprayed onto each lift.	Soil (vadose)	The system is designed to treat TCE, 1,2-DCE, and 1,2-DCA. Numerous other contaminants are also present. The concentration of all of the unidentified compounds decreased along with the concentrations of the regulated compounds.	Full-scale operations were completed on 12/30/96.	Gregory C. Weeks Inland Environmental, Inc. 3921 Howard St. Skokie, IL 60076 Inland@Inland-Env.com (847) 677-7500

* BFSS can be downloaded from the following Web site: <<http://www.clu-in.org/PRODUCTS/MOREINFO/Bfss.htm>>. BFSS is also available on diskette from EPA's Center for Environmental Research Information (CERI) at (513) 569-7562. The BFSS was queried in January 2000 to obtain pertinent data.

** Remediation efforts at this site also are included in Exhibit A-1 (Summary of Case Studies of *In Situ* Bioremediation Technology Applications) and Exhibit A-2 (Summary of Full-Scale Applications and Pilot Studies Identified in Proceedings of the Fifth International *In Situ* and On-Site Bioremediation Symposium).

¹ Contaminant Key: BTEX = benzene, toluene, ethylbenzene, and xylenes; DCA = dichloroethane; DCE = dichloroethene; PCB = polychlorinated biphenyl; PCE = tetrachloroethene; TCA = trichloroethane; TCE = trichloroethene

Case Study # 1
Aerobic Degradation Field Demonstration
at Moffett Naval Air Station, Mountain View, California

Summary Information [1,4,12]

Site Name, Location	Moffett Naval Air Station, Mountain View, CA
EPA ID Number	CA2170090078
Mechanism(s)	Aerobic Oxidation (Cometabolic and Direct)
Technology	Electron Acceptor Addition (Oxygen and Hydrogen Peroxide) Electron Donor Addition (Methane, Toluene, and Phenol)
Configuration	Groundwater Recirculation
Technology Scale	Field Demonstration
Media/Matrix Treated	Groundwater
Contaminants Targeted	TCE, cis-DCE, trans-DCE, VC
Period of Operation	September 1986 to November 1988 (methane addition studies)

Site History/Source of Contamination [1,4,6,7]

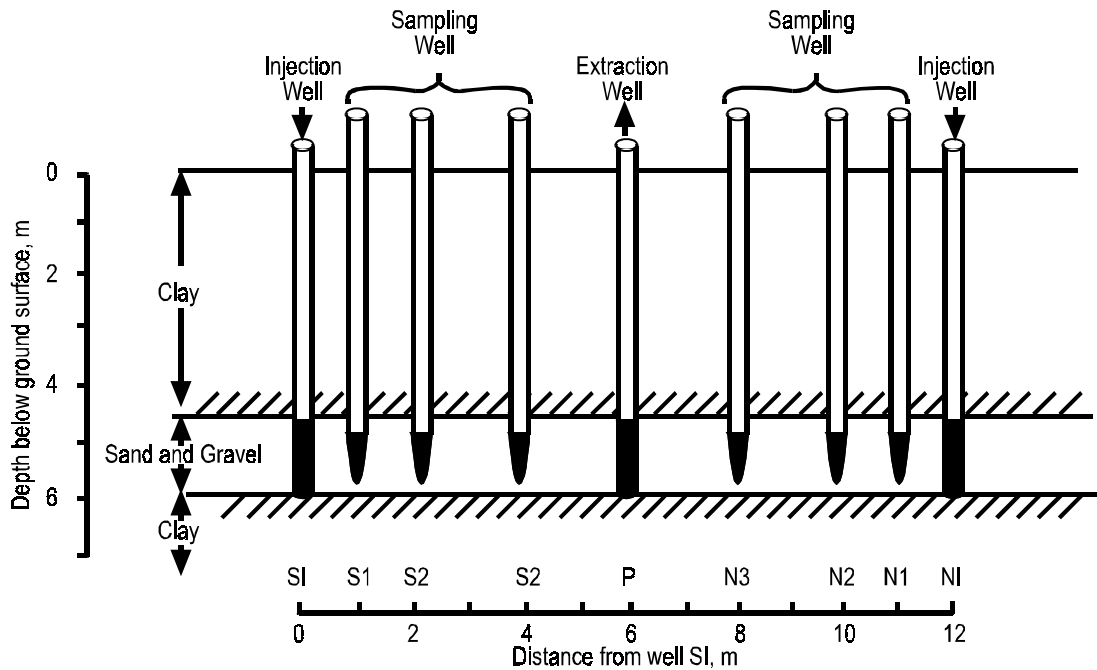
Moffett Naval Air Station (Moffett), used for aircraft operations and maintenance, operated from 1933 to 1994. In 1994, the Navy ceased operations and the airfield was transferred to the National Aeronautics and Space Administration (NASA). Moffett is located 35 miles south of San Francisco in Santa Clara County. Soil and groundwater at the site are contaminated with petroleum products and chlorinated aromatic hydrocarbons (CAHs) such as tetrachloroethene (PCE) and trichloroethene (TCE). Moffett is adjacent to other Superfund sites in the Middlefield-Ellis-Whisman (MEW) study area, and a large groundwater plume crosses Moffett from off-site sources. This site was added to the National Priorities List (NPL) on July 22, 1987 and is being addressed through Federal actions. Several Records of Decision (RODs) have been signed for this facility, including RODs for OU 1 (Sites 1 and 2 Landfills), dated August 1997; OU 2 (East Side Soils), dated December 1994; and OU 5 (East Side Aquifers), dated June 1996. In addition, for the West Side Aquifers, the Navy adopted an adjacent site's ROD, dated 1989.

Moffett was selected by researchers from Stanford University for a field demonstration of *in situ* aerobic degradation to treat groundwater contaminated with CAHs. A series of experiments was conducted between September 1986 and November 1998 to evaluate native bacteria enhanced through addition of methane, toluene, and phenol in degrading CAHs, including PCE and TCE.

Geology/Hydrogeology/Contaminant Characterization [3,5,11,12]

As shown in Figure 1, the demonstration site (test zone) was approximately 4 to 6 meters (m) below ground surface (bgs), located in a shallow, confined aquifer (1.5 m thick) consisting of sands and gravels. The groundwater velocity ranged from 1.5 to 3 m/day and the hydraulic conductivity of the aquifer was 0.11 cm/sec. In addition, indigenous methanotrophic bacteria were reported to be present in the aquifer.

Figure 1. Cross-section of Well Field Used at Moffett [4,5,12]



The CAHs present in the test zone prior to the demonstration included 1,1,1-trichloroethane (TCA) and 1,1-dichloroethane (DCA). However, TCE, cis-dichloroethene (cis-DCE), trans-dichloroethene (trans-DCE), and vinyl chloride (VC) were not detected in the groundwater in the test zone. As described below, regulatory approval was obtained to inject TCE, cis- and trans-DCE, and VC into the groundwater for the demonstration.

Matrix Characteristic	Value [11,12]
Soil Type	sand and gravel
Depth to Groundwater	4 to 6 m bgs
Thickness of Aquifer(s)	1.5 m
Fraction of Organic Carbon	0.00112 ± 0.00020
Hydraulic Conductivity	0.11 cm/sec
pH	6.5
DNAPL Present	None identified
Nitrogen	30 to 60 milligrams per liter (mg/L) (as nitrate)
Phosphorus	<0.1 mg/L (as phosphate)
Groundwater Velocity	1.5 to 3 m/day

Technology Description [4,5,7,12]

The demonstration of aerobic degradation was performed under induced-gradient conditions created by the extraction and injection of groundwater. As shown in Figure 1, groundwater was extracted at well P, amended chemically, and injected at wells SI and NI, located 6 m from extraction well P (information about the construction and operation of the wells was not provided). Regulatory approval was obtained for injecting TCE, cis- and trans-DCE, and VC into the groundwater.

Table 1 presents a summary of the nine experiments that were conducted over three seasons of the demonstration, including the period of operation, groundwater extraction and injection rates, chemical amendments, and processes studied. The experiments included biostimulation (Biostim) to stimulate the activity of native methane-using bacteria, and biotransformation (Biotran) to transform TCE into lower chlorinated compounds. Tracer experiments, using bromide, were performed to evaluate organic transport and “Decmeth” experiments were performed to evaluate methane addition.

Concentrations of CAHs, methane, DO, and bromide were monitored using the wells shown in Figure 1. An automated data acquisition and control system was used to provide as many as six sets of analyses per day at each of the sampling locations.

Additional experiments performed at the site included using phenol and toluene (alternative electron donors) as substrates in place of methane, and using hydrogen peroxide as an alternative to oxygen.

Table 1. Summary of Experimental Conditions Used at Moffett [4]

Experiment	Extraction (E) and Injection (I) Rates	Duration	Chemical Amendments (average mg/L)	Processes Studied
First Season				
Biostim 1	E: 8 L/min I: 1 L/min	9/5/86 - 9/30/86	Methane: 5.9 DO: 20.8 Bromide: 166	Biostimulation of native methane-using bacteria. Alternating pulse injection of methane and DO.
Biotran 1	E: 8 L/min I: 1 L/min	9/30/86 - 10/21/86	Methane: 5.7 DO: 22.2 TCE: 0.097	Biotransformation of TCE with active biostimulation. Nonsteady-state conditions.
Biotran 4	E: 8 L/min I: 1 L/min	12/10/86 - 12/31/86	Methane: 5.2 DO: 23 Bromide: 159 TCE: 0.051	Biotransformation of TCE with active biostimulation. Steady-state conditions.
Second Season				
Tracer 8	E: 10 L/min I: 1.5 L/min	7/6/87 - 8/15/87	DO: 14.3 Bromide: 78 TCE: 0.048 cis-DCE: 0.110 trans-DCE: 0.112	Transport and breakthrough of bromide, TCE, cis- and trans-DCE without biostimulation.

Table 1. Summary of Experimental Conditions Used at Moffett [4] (continued)

Experiment	Extraction (E) and Injection (I) Rates	Duration	Chemical Amendments (average mg/L)	Processes Studied
Bioestim 2	E: 10 L/min I: 1.5 L/min	8/17/87 - 10/26/87	Methane: 5.3 DO: 23.4 Bromide: 44 TCE: 0.036 cis-DCE: 0.091 trans-DCE: 0.092	Simultaneous biostimulation and biotransformation of TCE, cis- and trans-DCE.
Decmeth 1	E: 10 L/min I: 1.5 L/min	10/27/87 - 11/8/87	DO: 24.5 TCE: 0.045 cis-DCE: 0.136 trans-DCE: 0.095	Test if active biotransformation occurs without addition of methane.
Third Season				
Tracer 11	E: 10 L/min I: 1.5 L/min	8/10/88 - 10/10/88	Bromide: 72 TCE: 0.047 cis-DCE: 0.085 trans-DCE: 0.050	Transport and breakthrough of bromide, TCE, cis- and trans-DCE without biostimulation.
Tracer 12	E: 10 L/min I: 1.5 L/min	10/10/88 - 10/20/88	Bromide: 44 TCE: 0.042 cis-DCE: 0.100 trans-DCE: 0.054 VC: 0.044	Transport and breakthrough of bromide and VC while continuing injection of TCE, cis- and trans-DCE.
Bioestim 3	E: 10 L/min I: 1.5 L/min	10/20/88 - 11/23/88	Methane: 6.6 DO: 21.3 Bromide: 45 TCE: 0.046 cis-DCE: 0.100 trans-DCE: 0.052	Simultaneous biostimulation and biotransformation of TCE, cis- and trans-DCE, and VC.

Technology Performance [2,3,4,7,12]

The objective of the field demonstration was to collect data to be used in evaluating aerobic degradation of CAHs under several different experimental scenarios. Specific remedial goals were not established for this demonstration.

Several methods were used to evaluate the amount of CAHs that were biodegraded in these experiments, including mass balances on the amounts of CAH injected and extracted, and comparison of breakthrough concentrations using controlled experiments and bromide tracers. Results showed that active use of methane in the treatment zone was required for biodegradation of CAHs, and that groundwater residence times in the treatment zone of 1-2 days resulted in biodegradation of TCE at 20 - 30%, cis-DCE at 45 - 55%, trans-DCE at 80 - 90%, and VC at 90- 95%. The results indicated a similar degree of biodegradation of TCE over the three seasons of field testing, suggesting that there was no apparent increase in the ability of the bacteria to degrade TCE. In addition, results showed that an intermediate biotransformation product, trans-DCE oxide, was produced in a manner consistent with the expected transformation pathway for trans-DCE. Detailed analytical results for each of the nine experiments are provided in reference 4 for this case study.

Table 2 summarizes the results from the third season of the methane addition experiments, and the experiments with phenol and toluene as primary substrates. As shown in the table, the use of phenol and toluene achieved higher percent removals of TCE (93 - 94%) compared with use of methane (19%).

Table 2. Summary of Results (% Removal) Using Different Substrates at Moffett [3]

Primary Substrate	Substrate Concentration (mg/L)	% Removal by Constituent				
		TCE	1,1-DCE	cis-DCE	trans-DCE	Vinyl Chloride
Methane (third season)	6.6	19	NE	43	90	95
Phenol	12.5	94	54	92	73	>98
Toluene	9	93	NE	>98	75	NE

Note:
NE - not evaluated

Hydrogen peroxide was found to achieve TCE removals similar to those achieved using oxygen. While 1,1-DCE was partially transformed in the study with phenol, the transformation products were found to be toxic to the transforming bacteria.

Additional information and discussion about the experiments conducted using phenol and toluene are provided in references 8 - 10 for this case study.

No exceptions to established quality assurance/quality control (QA/QC) protocols were noted in the available information.

Technology Cost

No information was provided about the cost for the *in situ* bioremediation treatment system used at Moffett.

Summary Observations and Lessons Learned [3,4,7-12]

The results of the field demonstration at Moffett showed that native bacteria enhanced with methane, phenol, or toluene, plus oxygen or hydrogen peroxide was effective in degrading CAHs in groundwater. Concentrations of CAHs were reduced by as much as 94% for TCE, 92% for cis-DCE, and 98% for VC. Native bacteria enhanced with phenol and toluene achieved higher removal rates for TCE than bacteria enhanced with methane. The results from the field experiments were consistent with the results from batch soil column laboratory testing using aquifer solids from the test zones.

The presence of 1,1-DCE in the groundwater was found to be toxic to the bacteria, and should be considered when evaluating this technology for use in other applications. However, the relatively low concentration of phosphate in the groundwater did not limit the biodegradation of CAHs at this site. According to the researchers, other phosphate minerals may have dissolved in the groundwater to replenish this mineral as it was being removed by the bacteria.

During the field demonstration, the use of alternating pulsed addition of methane and oxygen minimized biofouling in the area near the injection well.

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Case Study # 2
Aerobic Degradation Field Demonstration
at Site 19, Edwards Air Force Base, California

Summary Information [1,2,3]

Site Name, Location	Edwards Air Force Base, CA
EPA ID Number	CA1570024504
Mechanism(s)	Aerobic Oxidation (Cometabolic and Direct)
Technology	Electron Acceptor Addition (Oxygen and Hydrogen Peroxide) Electron Donor Addition (Toluene)
Configuration	Groundwater Recirculation
Technology Scale	Field Demonstration
Media/Matrix Treated	Groundwater
Contaminants Targeted	TCE
Period of Operation	February 5, 1996 to April 1, 1997

Site History/Source of Contamination [3,7]

Edwards Air Force Base (AFB), located on the western portion of the Mojave Desert, about 60 miles north of Los Angeles, covers approximately 301,000 and is used for aircraft research and development. From 1958 through 1967, engines for the X-15 rocket airplane were maintained in facilities at the site, and trichloroethene (TCE) was used to clean the engines. The used TCE was disposed of at Site 19, an area of about 53 acres on the west side of Rogers Dry Lake, resulting in groundwater contamination. The contaminant plume extends approximately 3,200 ft down-gradient from the contamination source, and nearly the same distance cross-gradient. This site was added to the National Priorities List (NPL) on August 30, 1990, and is being addressed through Federal actions. A Record of Decision (ROD) had not been signed for this facility at the time of this report.

A field demonstration of aerobic biodegradation was performed at Site 19. The area of the plume used for this field demonstration was about 400 meters (m) east of the contamination source.

Geology/Hydrogeology/Contaminant Characterization [3,8,9,10]

The Site 19 demonstration area contains two relatively homogeneous aquifers. The upper, unconfined aquifer is 8 m thick, and is separated by a 2 m aquitard from the lower confined aquifer. The lower, confined aquifer is approximately 5 m thick and lies above weathered bedrock. At the demonstration site, the concentration of TCE in the groundwater plume varied between 500 and 1,200 micrograms per liter ($\mu\text{g/L}$), with average TCE concentrations in the upper and lower aquifer of 680 and 750 $\mu\text{g/L}$, respectively. No 1,1-DCE was found at the site prior to the demonstration.

Matrix Characteristic	Value
Soil Type	fine to medium sized sand mixed with some silt
Depth to Groundwater	9 m below ground surface (bgs)
Thickness of Aquifer(s)	15 m (total)
Fraction of Organic Carbon	0.0001 to 0.0004
DNAPL Present	None identified
Hydraulic Conductivity	1.5 to 5.5 x 10 ⁻³ cm/sec to east/southeast (average 3.4 x 10 ⁻³ cm/sec)
Groundwater Velocity	6.9 cm/day

Technology Description [3,4,6,8]

The *in situ* bioremediation treatment system used at this site, shown in Figure 1, was designed based on the results from the demonstration at Moffett (case study No. 1), and consisted of two 8-in diameter, PVC treatment wells installed approximately 24 m deep and spaced 10 m apart. Each treatment well was screened in both the upper and lower aquifers (15 m and 10 m, respectively), and a submersible pump, placed in each well, was used to draw contaminated water into the well through one of the screens. The initial flow rate for the wells was 38 liters per minute (L/min) to limit drawdown in the upper aquifer and pressure changes in the lower aquifer. The primary substrate (toluene) and oxygen were introduced into the wells via feed lines and mixed with the water using static mixers inside the wells. The groundwater, containing TCE, toluene, and oxygen, was discharged from the second screen into the aquifer, where a treatment zone developed around the well. Treatment well 1 (T1) withdrew water from the upper aquifer and discharged it into the lower aquifer, while treatment well 2 (T2) withdrew water from the lower aquifer and discharged it into the upper aquifer. This process recirculated the water between the two aquifers creating a bioreactive treatment cell.

Treatment system operation included groundwater pumping, pulsed addition of toluene, and addition of dissolved oxygen (DO, as gaseous oxygen) and hydrogen peroxide (H₂O₂). The system was operated for 444 days. The demonstration included five phases, during which time the operating parameters were varied as follows:

- (1) pre-operational studies (days 0 - 33)
- (2) establishment of a toluene-degrading consortium (days 34 - 55)
- (3) pre-steady-state operation (days 56 - 136)
- (4) steady-state operation (days 142 - 271)
- (5) balanced flow operation (days 317 - 444)

Operating parameters for steady-state and balanced flow periods, which correspond to results provided below, are shown in Tables 1 and 2.

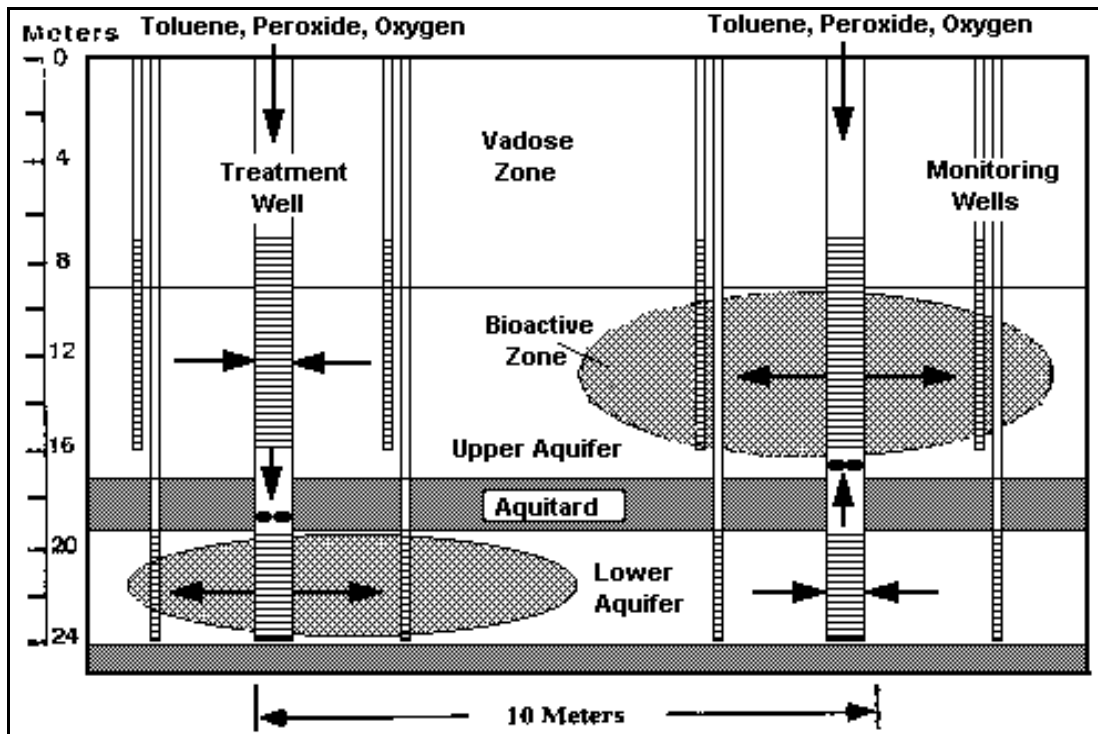
Table 1. Operating Parameters for Steady-State Operation (Days 142 - 271) [3]

Treatment Well	Groundwater Pumping Rate (L/min)	Toluene Addition (mg/L)	Toluene Addition - Pulses per Day	DO Addition (mg/L)	H ₂ O ₂ Addition (mg/L)
T1	25	0 - 11.6	0.67 - 1	44	17 - 117
T2	38	13.4	1	29	47 - 63

Table 2. Operating Parameters for Balanced Flow Operation (Days 317 - 444) [3]

Treatment Well	Groundwater Pumping Rate (L/min)	Toluene Addition (mg/L)	Toluene Addition - Pulses per Day	DO Addition (mg/L)	H ₂ O ₂ Addition (mg/L)
T1	25	9.0	0.67	44	47
T2	25	9.0	0.67	44	47

Figure 1: Cross-section of two-well treatment system used at Edwards AFB [3]



Technology Performance [2,8,9,10]

The objectives of the pilot study at Edwards AFB were to evaluate the advantages and limitations of *in situ* bioremediation for full-scale aquifer remediation. Specific remedial goals (contaminant concentrations in groundwater) were not established for the demonstration.

An area of 480 m² (0.12 acres) was monitored using 20 monitoring wells. Fourteen of the monitoring wells surrounded treatment wells T1 and T2 in a diamond formation, and two wells were nested between the treatment wells. Other wells were located at the “compass points” (North, South, East, West) surrounding the site. The 14 diamond formation wells and three of the four compass point wells were screened in both the upper and lower aquifers, allowing sampling from each aquifer independently. 10,500 samples were collected and analyzed automatically at the site throughout the course of the demonstration.

Comparison of measured TCE concentrations at the treatment well discharge screens, and at monitoring wells located 7.5 m away from the screens, allowed estimation of TCE removal in the bioactive treatment zones surrounding the discharge screens. The results from these analyses, during steady-state and balanced flow operation, are presented in Table 3.

Table 3. TCE Concentrations During Steady-state and Balanced Flow Operation [3]

Treatment Well - Aquifer	Operating Period (Days)	Average TCE Concentration in Treatment Well (µg/L)	Average TCE Concentration in Monitoring Well 7.5 m Distant (µg/L)	TCE Removal % (average and standard deviation)
T1 - lower	145 - 204	80	17	79 ± 42
T1 - lower	212 - 271	63	26	59 ± 22
T1 - lower	365 - 444	107	18	83 ± 16
T2 - upper	145 - 204	304	46	85 ± 9
T2 - upper	212 - 271	254	29	89 ± 7
T2 - upper	365 - 444	171	24	86 ± 9

Table 3 shows that the average reduction of TCE during steady-state operation (days 145 - 271) was 87% in the upper aquifer bioactive zone and 69% in the lower aquifer adjacent to treatment well T1 discharge screen. During balanced flow operation (days 365 - 444), the average removal of TCE was 86% and 83% in the upper and lower aquifer bioactive zones, respectively. Over the duration of the demonstration, TCE concentrations were reduced by 97.7%, from 1,150 µg/L (groundwater moving into the study area) to 27 µg/L (groundwater moving out of the study area) and toluene removal generally exceeded 99.98%. According to the researchers the overall TCE concentration reduction of 97.7% is higher than the removals reported in Table 3 as groundwater recirculated through the bioactive zone multiple times during the overall demonstration. The dual-well system was found to be technically feasible for remediation of TCE in a two aquifer system.

No information was provided about potential degradation products from this demonstration. The researchers presumed that toluene degraded aerobically to carbon dioxide and water, and TCE was cometabolized, ultimately producing carbon dioxide, water, and chloride ions. No exceptions to established quality assurance/quality control (QA/QC) protocols were noted in the available information.

Technology Cost [5]

Table 4 provides the actual cost for the *in situ* bioremediation treatment system used for the demonstration at Edwards AFB, including capital and operation and maintenance costs. Software is available [5] for estimating costs of applying this technology at a site with specified characteristics. These actual costs are provided as an example in the software user’s guide.

Table 4. Actual Costs for the Field Demonstration at Edwards AFB [5]

Cost Element	Actual Cost (1995-1996 \$)
Capital - Treatment wells (2)	30,000
Capital - Other treatment equipment - flow sensors and controllers, static mixers, packing assembly, deionized water system, pumps and ancillary equipment, tubing and connectors, valves and fittings)	32,707
Capital - Monitoring wells	190,000
Capital - Monitoring equipment (pumps and ancillary equipment, tubes and connectors, valves and fittings, miscellaneous supplies)	70,746
Total Capital Costs	323,453
Annual O&M - Materials - Well Redevelopment (\$4,000/well-year x 2 wells)	8,000
Annual O&M - Materials - Hydrogen Peroxide, 30%	4,633
Annual O&M - Materials - Toluene	47
Annual O&M - Materials - Oxygen Gas	1,674
Total Annual O&M Costs	14,354

Volume of Water in Test Area	1,160 m ³
Volume of Water Pumped	12,132 m ³ from upper to lower aquifer 16,063 m ³ from lower to upper aquifer

Summary Observations and Lessons Learned [3]

The dual-well system met the objectives of the pilot study, and was found to be technically feasible for remediation of TCE in a two aquifer system. In addition, this technology might be feasible for use in a single aquifer system where low permeability layers separate lower and upper zones, and where vertical hydraulic conductivity is significantly lower than horizontal conductivity. Alternatively, with a relatively homogeneous single aquifer system, groundwater might be pumped to the surface from one location and then reinjected at another location with chemical amendments added at the surface or down-well at the injection location.

Prevention of well clogging was identified as an important operational consideration for application of this technology. To control well clogging during this demonstration, site operators used well redevelopment (three times in the upper and twice in the lower aquifer) and addition of hydrogen peroxide, which increased the operational costs.

The extensive network of monitoring wells was a major capital cost component for this application. The monitoring system was installed to allow a detailed evaluation of the treatment system's performance. Monitoring of this magnitude would likely not be required for a full-scale application.

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Case Study #3
Methane Enhanced Bioremediation Field Demonstration Using Horizontal Wells
at Savannah River Site, Aiken, South Carolina

Summary Information [1,4]

Site Name, Location	Savannah River Site, Aiken, SC
EPA ID Number	SC1890008989
Mechanism(s)	Aerobic Oxidation (Cometabolic and Direct)
Technology	Nutrient Addition Electron Acceptor Addition (Oxygen) Electron Donor Addition (Methane)
Configuration	Direct Injection
Technology Scale	Field Demonstration
Media Treated	Sediment and Groundwater
Contaminants Targeted	TCE, PCE
Period of Operation	February 26, 1992 to April 30, 1993

Site History/Source of Contamination [1,4,5]

The U.S. Department of Energy (DOE), Savannah River Site (SRS) is a 300 square mile facility located in Aiken, South Carolina that has been used for a wide range of operations associated with the research and production of nuclear materials. Area M at the facility was used for aluminum forming and metal finishing operations. From the 1950's to the 1980's, wastewaters from Area M operations were discharged to an unlined settling basin and a nearby stream, resulting in soil and groundwater in the area becoming contaminated with high levels of chlorinated solvents, primarily trichloroethylene (TCE) and tetrachloroethene (PCE). Dense nonaqueous phase liquids (DNAPLs) have also been observed. In September 1985, a full-scale pump and treat system began operating at the site. This site is was added to the National Priorities List on November 21, 1989. A Record of Decision (ROD) had not been signed for this facility at the time of this report.

DOE, as part of the volatile organic compound (VOCs) in Non-Arid Soils Integrated Demonstration program, tested several innovative technologies to augment the pump and treat system in Area M. This report focuses on the field demonstration of methane enhanced bioremediation using horizontal wells. The demonstration site was located within the VOC groundwater plume, estimated to cover about 1200 acres and to be about 150-ft thick. Prior to the demonstration, concentrations of TCE and PCE in groundwater ranged from 10 to 1,031 ug/L and 3 to 124 ug/l, respectively. Sediment TCE and PCE concentrations ranged from 0.67 to 6.29 mg/kg and 0.44 to 1.05 mg/kg, respectively.

Geology/Hydrogeology/Contaminant Characterization [1,5]

The demonstration area was underlain by relatively permeable sands with thin lenses of clayey sediments. The clay layers generally were relatively thin and discontinuous, with thicker clay layers found at depths of 90 and 160 feet below ground surface (bgs). The water table occurred at depths

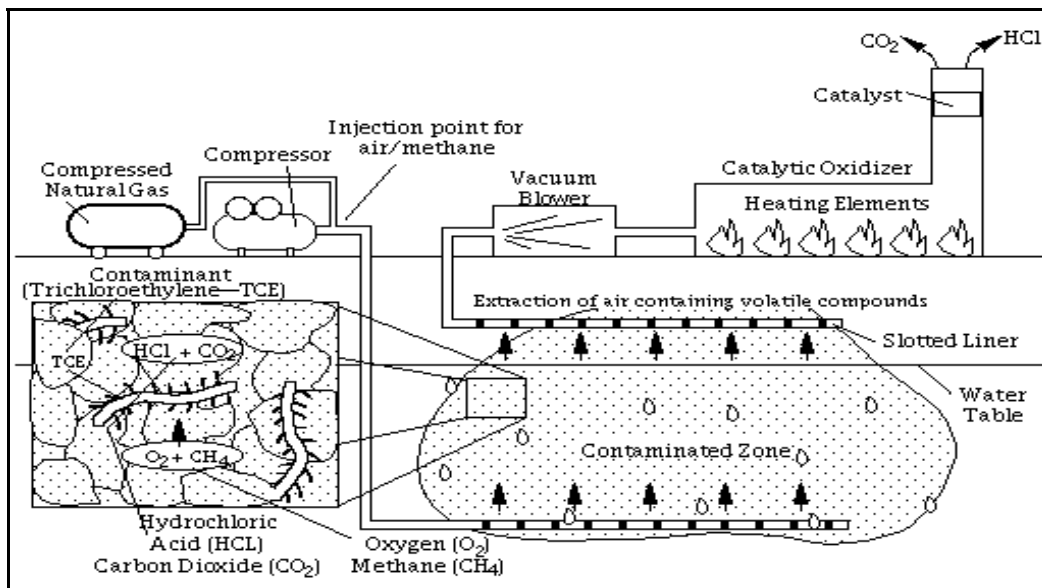
ranging from 120 and 135 feet bgs. The groundwater flow was radial, extending outward from a groundwater plateau under the demonstration area. In addition, there was a moderate downward gradient beneath the site, with vertical flow rate estimated to be 2 to 8 feet/year.

Matrix Characteristic	Value
Soil Type	sand, clay, and gravel
Depth to Groundwater	ranges from 120 to 135 feet bgs
Thickness of Aquifer(s)	150 feet
DNAPL Present	None identified
Groundwater Velocity	15 to 100 feet/year (horizontal)

Technology Description [1,2,3]

Figure 1 presents a process schematic of the methane enhanced bioremediation (MEBR) system used for the demonstration at the M area. The system included two horizontal wells. The “lower” horizontal well was placed below the water table (saturated zone) at a depth of 175 feet bgs, with a screen length of 310 feet. The “upper” horizontal well was placed in the vadose zone at a depth of 80 feet bgs, with a screen length of 205 feet. Air and gas were injected into the saturated zone through the lower horizontal well at a rate of 200 scfm. Air and contaminants were then extracted from the vadose zone through the upper horizontal well at a rate of 240 scfm. A thermal catalytic oxidizer, operated at 825° C, was used to treat the extracted vapors, prior to discharge to the atmosphere.

Figure 1: Methane Enhanced Bioremediation (MEBR) [1]



The demonstration was performed in six different operational modes, as described in Table 1. These included baseline tests of the vapor extraction and injection systems, a series of nutrient additions, a tracer test, and an assessment of microbiological assays for monitoring performance.

Table 1. Modes for the Demonstration [1,3]

Operational Mode	Description of system operation
Baseline Test	Initial vacuum extraction of vadose zone gases at a rate of 240 scfm
Baseline Test	Addition of air sparging - simultaneous injection of air into the saturated zone coupled with vacuum extraction of the vadose zone at a rate of 202 scfm (84% of the first baseline test)
Nutrient Addition -1	Addition of 1% methane
Nutrient Addition - 2	Addition of 4% methane
Nutrient Addition - 3	Pulsed 4% methane addition at a rate of 8 hr every two days
Nutrient Addition - 4	Continuous addition of a combination of nitrous oxide at 0.007% and triethyl phosphate at 0.07% in air in combination with pulses of 4% methane
Tracer tests	Helium tracer tests to measure the amount of injected methane consumed by the indigenous microbes
Microbiological Assays	Comparison of microbial assays for monitoring and control of <i>in situ</i> bioremediation

Technology Performance [1,2,3]

After 384 days of operation, concentrations of PCE and TCE in sediments were reduced to below detectable limits, and concentrations of PCE and TCE in groundwater were reduced to below 5 ppb each for PCE and TCE. In addition, soil gas concentrations decreased by more than 99%. The system removed about 17,000 lbs of VOCs through a combination of vacuum extraction and biodegradation. The concentration of TCE and PCE in the sediments before and after the demonstration were used to calculate the mass of VOCs degraded. The vacuum component of the system removed 12,096 lbs of VOCs and the biological component degraded 4,838 lbs of VOCs.

The addition of methane stimulated the growth of methanotrophs. During the 1% methane addition phase, the population of methanotrophs increased by several orders of magnitude, to levels close to 100,000 MPN/ml. During the 4% methane addition phase, the population of methanotrophs increased initially, then decreased as a result of nutrient depletion. The addition of nitrogen and phosphorous nutrients with pulsed methane stimulated microbial activity, and was reported to have optimized bioremediation and mineralization of TCE and PCE in groundwater and sediments. The results of the helium tracer tests indicated that more than 50% of the injected methane was consumed by indigenous microbes before it reached the extraction well. No results were provided from the microbiological assays.

The zone of influence of the extraction well in the vadose zone was reported to be greater than 200 feet based on pressure measurements. The sparge zone of influence in the saturated zone, measured using electrical resistance tomography, was reported to be a “complex three-dimensional network of channels”

extending as far as 100 feet from the injection well. The system was operational 90% of the time and no problems were reported during the demonstration.

Technology Cost [1,5,6]

Table 2 presents the projected costs for full-scale application of MEBR. The projected capital costs were \$452,407 (including equipment costs amortized over 10 years, and costs for well installation and mobilization), and the projected operation and maintenance (O&M) costs were \$236,465 (including monitoring, consumables, and demobilization).

Table 2. Project Costs for Full-scale MEBR Application [1,5]

Element	Cost (\$)
Capital	
Site cost	5,400
Equipment cost	9,200
Design and Engineering	10,000
Mobile equipment	18,000
Well Installation	183,000
Other fixed equipment	183,732
Mobilization	43,075
Total Capital Equipment and Mobilization Cost	452,407
O&M Costs	
Monitoring/maintenance	71,175
Consumables	122,215
Demobilization	43,075
Total O&M	236,465

Summary Observations and Lessons Learned [1,2]

The *in situ* bioremediation system demonstrated as SRS removed about 17,000 lbs of VOCs through vacuum extraction (about 12,000 lbs) and through biodegradation (about 5,000 lbs). According to DOE, the addition of nitrogen and phosphate nutrients in conjunction with 4% pulsed methane provided the best results of the four nutrient addition campaigns tested.

No toxic intermediates were produced during the demonstration. However, the use of technical grade methane was found to be growth inhibiting because it contained small amounts of acetylene which is poisonous to the microbes.

Los Alamos National Laboratory completed a cost-benefit analysis that showed that *in situ* bioremediation could reduce costs by more than 30% compared to a baseline technology of SVE/pump

and treat. According to DOE, *in situ* bioremediation could reduce the time required to remediate a site by 5 to 7 years compared to SVE/pump and treat.

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Case Study #4
Full Scale Enhanced Bioremediation at the Texas Gulf Coast Site,
Houston, Texas

Summary Information [1,2]

Site Name, Location	Texas Gulf Coast Site, Houston, Texas (actual site name confidential)
EPA ID Number	Not available
Mechanism(s)	Anaerobic Reductive Dechlorination (Cometabolic and Direct)
Technology	Nutrient Addition Electron Donor Addition (Methanol)
Configuration	Groundwater Recirculation
Technology Scale	Full
Media/Matrix Treated	Groundwater
Contaminants Targeted	TCE, cis-1,2-DCE, VC
Period of Operation	Ongoing (data available from June 1995 to December 1998)

Site History/Source of Contamination [1,2,3]

The Texas Gulf Coast site (actual site name confidential) is an abandoned industrial manufacturing facility located near Houston, Texas that operated between 1952 and 1985. Trichloroethene (TCE) was used in facility operations until about 1978. In 1986, elevated levels of TCE were found in groundwater at the site, and groundwater monitoring has been performed periodically since that time. Groundwater at the site is being remediated through the State of Texas Voluntary Cleanup Program, administered by the Texas Natural Resource Conservation Commission (TNRCC).

Monitoring data from 1986 to 1995 showed that TCE concentrations in the groundwater had decreased from approximately 50 to 22 mg/L, and that TCE degradation products such as DCE were present in the groundwater, indicating that natural attenuation was occurring at the site. In 1995, an enhanced bioremediation system was installed to actively remediate the contaminated groundwater at the site to a point where natural attenuation would prevent further migration of the plume. The TNRCC approved the work plan for the use of *in situ* bioremediation at the site and for reinjection of water under the Underground Injection Control (UIC) program.

Geology/Hydrogeology [1,3]

The area of groundwater contamination is approximately 600 ft by 700 ft, located in an unconsolidated aquifer which occurs at a depth of approximately 12 - 20 ft below ground surface (bgs). The upper aquifer is underlain by a 15-ft thick clay layer, below which lies a deeper aquifer, which is not contaminated. The natural flow direction for the upper aquifer is to the southeast. A flood control ditch is located on the eastern edge of the area, and intercepts the upper two feet of the aquifer.

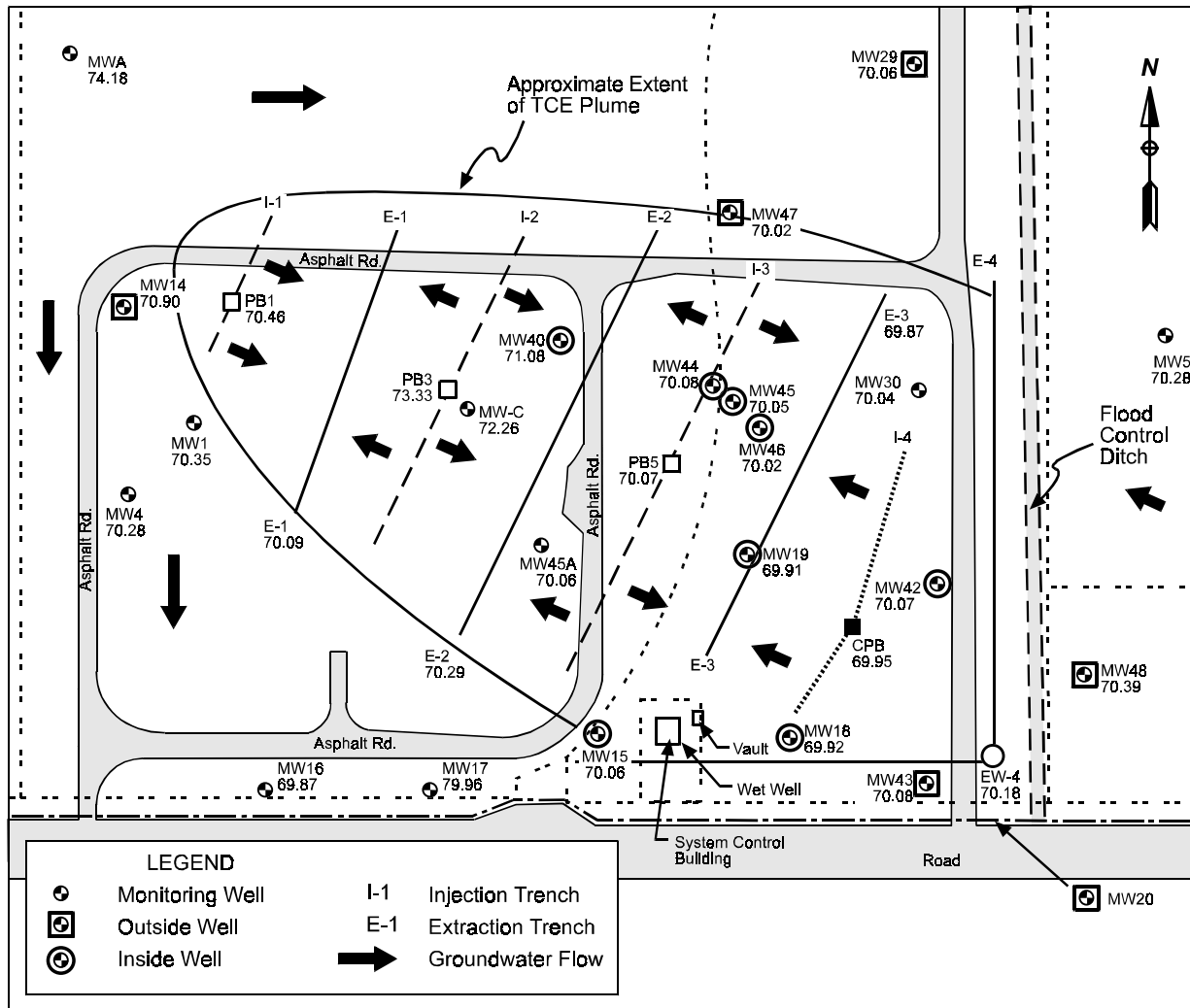
Matrix Characteristic	Value
Soil Type	Silty sand
Depth to Groundwater	12 - 20 ft bgs
Thickness of Aquifer	Approximately 8 ft
Fraction of Organic Carbon	Not provided
DNAPL Present	None identified
Hydraulic Conductivity	1×10^{-4} to 4×10^{-4} cm/sec
pH	Approximately 6.7 to 7.2
Nitrogen	<0.1 mg/L
Sulfur (as Sulfate)	Approximately 30 mg/L
Groundwater Velocity	4 - 18 ft/yr

Technology Description [1,3]

The *in situ* bioremediation treatment system being used at this site consists of an alternating series of four extraction (1,800 linear ft total) and four injection (1,100 linear ft total) trenches set at a spacing of approximately 100 ft, as shown in Figure 1. The furthest down-gradient trench is an extraction trench that runs along the portion of the down-gradient property line intersected by the plume.

The extraction trenches were completed to a depth of at least one foot into the bottom clay layer (20 - 22 ft bgs), and were sloped to a sump. A perforated pipe was installed along the bottom of each trench and the trenches were filled with gravel. The injection trenches were constructed in a manner similar to that used for the extraction trenches; however, the perforated pipes were installed at a depth of approximately 10 ft bgs. Extracted groundwater is pumped to a holding tank in the control building. Water drains from the holding tank to a wet well and then to the injection trenches. The above-ground equipment was designed to minimize the introduction of air into the equipment.

Figure 1: System Layout, Extent of TCE Plume, and Groundwater Flow Directions for Texas Gulf Coast Site [1,3]



System operation began in August 1995 with a circulation rate of 12 gallons per minute (gpm). Addition of nitrogen and phosphorus nutrients began in September 1995. In June 1996, methanol was added, along with nitrogen and phosphorus, to serve as a primary substrate and to further reduce the dissolved oxygen and oxidation-reduction potential to levels that were thought to be more favorable to anaerobic degradation of TCE. Nutrient addition was discontinued in May of 1997 because it appeared to be preventing continued decrease in the redox potential within the treatment area; methanol addition continued. As of January 1999, the recirculation rate averages 6 to 8 gpm, and a total of 12 million gallons have been recirculated through the system (approximately 2.5 pore volumes). System operating parameters are summarized in Table 1.

Table 1. Operating Parameters for the Enhanced Bioremediation System [1,3]

Parameter	Value
Circulation Rate	12 gpm (August 1995 - January 1999) 6 to 8 gpm (starting in January 1999)
Methanol Concentration	up to 500 mg/L (in January 1999)
Nitrate Concentration	initially 9 mg/L (as potassium nitrate) (discontinued in May of 1997)
Phosphate Concentration	initially 9 mg/L (as potassium tripolyphosphate) (discontinued in May of 1997)

Technology Performance [1,2,3]

The performance goals of this treatment system are to achieve stable or declining contaminant concentrations and to remediate the groundwater to a point where natural attenuation can be used to prevent future migration of the contaminant plume. Once these goals have been achieved, use of active bioremediation will be discontinued and groundwater at the site will be monitored for a period of 2 to 5 years to determine that the plume has not migrated. No specific cleanup goals have been identified for groundwater at this site.

Table 2 summarizes groundwater monitoring data from June 1995 (prior to system operation) to December 1998 for five sampling events, including data for TCE, DCE, and VC, as well as other parameters such as chloride, DO, and redox potential. The data presented are the average concentrations measured in six wells located outside of the treatment zone (“Outside Wells”) and in eight wells located within the treatment zone (“Inside Wells”), including one of the monitoring wells, well MW-40, that was located within the apparent “source” area. The average concentrations for the TCE, DCE, and VC in the “Inside Wells” were calculated two ways - one including the data from well MW-40, and one excluding the data from well MW-40.

Results for the inside wells, including the source area well MW-40, show the average concentration of TCE was reduced by about 88% (22.7 mg/L to 2.6 mg/L) and DCE by about 96% (1.91 mg/L to 0.682 mg/L). VC concentrations in the inside wells remained essentially unchanged (0.102 mg/L to 0.105 mg/L). When the results for well MW-40 are not included, the average concentration of TCE was reduced by about 99% (11.8 mg/L to 0.12 mg/L); DCE by about 87% (1.28 mg/L to 0.165 mg/L); and VC by about 30% (0.078 mg/L to 0.054 mg/L).

According to the site contractor, Well MW-40, located within the treatment area, has shown consistently elevated concentrations of TCE. Recent excavation from within this area identified a potential source of continuing release to the groundwater that was preventing the rate of decrease observed in the remaining plume. Soil in the potential source area was excavated during October 1998 to allow volatilization of the chlorinated organic compounds. Debris (concrete, piping, and trash) was excavated in this area; however, no evidence of dense non-aqueous phase liquids (DNAPL) was identified. In addition, TCE concentrations in portions of the plume have now decreased to below the detection limit (0.005 mg/L).

**Table 2. Summary of Technology Performance Data
Average Concentrations (mg/L) [3]**

Parameter	Analytical Methods*	Jun-95		May-96		Jun-97		Mar-98		Dec-98	
		Inside Wells	Outside Wells	Inside Wells	Outside Wells	Inside Wells	Outside Wells	Inside Wells	Outside Wells	Inside Wells	Outside Wells
Average including potential source well MW-40											
TCE	EPA 8260	22.7	0.070	16.7	0.07	4.02	0.748	2.80	0.046	2.60	0.065
cis-1,2-DCE	EPA 8260	1.91	< 0.005	1.0	< 0.005	1.31	0.049	2.90	0.006	0.682	0.009
Vinyl Chloride	EPA 8260	0.102	< 0.005	< 0.005	< 0.005	0.084	< 0.002	0.222	< 0.002	0.105	< 0.002
Average excluding potential source well MW											
TCE	EPA 8260	11.8	-	9.68	-	3.24	-	2.42	-	0.119	-
cis 1,2-DCE	EPA 8260	1.28	-	0.733	-	1.38	-	2.52	-	0.165	-
Vinyl Chloride	EPA 8260	0.078	-	0.016	-	0.095	-	0.181	-	0.054	-
Chloride	EPA 325.3	162	35	147	29	132	20	136	23	120	14
NO3-NO2	EPA 353.2	< 1.0	< 1.0	11	0.90	0.20	0.13	< 0.10	< 0.10	< 0.10	< 0.10
O-PO4	EPA 365.2	0.24	0.09	2.1	0.40	1.9	0.52	0.56	0.12	0.65	0.10
TKN	EPA 351.2	< 1.0	< 1.0	3.6	1.6	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Ferrous Iron	EPA 6010	-	-	-	-	0.23	< 0.05	0.27	< 0.02	2.1	< 0.02
Total Iron	EPA 6010	-	-	-	-	-	-	3.4	0.25	4.4	0.07
Sulfate	EPA 375.4	-	-	-	-	170	28	146	24	42	38
Sulfide	EPA 376.1	-	-	-	-	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Methane	EPA 3810	-	-	-	-	0.69	0.007	2.8	0.009	3.6	0.85
Ethene	EPA 3810	-	-	-	-	-	-	-	-	0.09	0.02
DO		1.7	3.4	1.1	2.4	0.45	1.6	0.65	1.6	1.1	2.3
Redox (mV)		+292	+319	+111	+126	+85	+178	-61	+332	-116	+245
pH (SU)		6.56	6.75	6.69	6.84	6.46	6.83	6.38	6.74	6.85	7.23

Notes: (-) Not Analyzed.
(*) Latest sampling event only.

The concentration of chloride was used to evaluate the potential impact of dilution on groundwater quality. As shown on Table 2, there is a difference between the chloride concentrations in the inside wells and those in the outside wells. According to the site contractor, assuming the chloride is due exclusively to dechlorination of TCE (and its degradation products), the average TCE concentration at project outset was estimated to be as high as 100 mg/L to 125 mg/L. During the active treatment period, chloride in the outside wells has decreased from approximately 35 mg/L to 14 mg/L. The site contractor applied the same assumptions about dilution to the treatment area and estimated that the December 1998 chloride concentration for the inside wells was approximately 65 mg/L. The differences between this and the measured concentration (120 mg/L) was assumed by the site contractor to be due to additional contributions from dechlorination of TCE (and the degradation products). Accounting for dilution, the site contractor reported that TCE concentrations were reduced by approximately 2% per month during the period of nutrient-only addition, and approximately 10% per month during the period of nutrient and methanol (substrate) addition. Also, the ratio of cis-1,2-DCE to TCE increased from approximately 0.06:1 to 0.30:1 after addition of methanol, suggesting more active dechlorination associated with higher concentrations of substrate.

According to the site contractor, current plans are to shut down the active bioremediation system by the middle of 1999. Should this occur, this site will meet its design goal of completing the active remediation period within 3-5 years (actual period of operation would be 4 years). It is anticipated that the approval for system shutdown will be based on the following:

- The impacted aquifer has been designated as “low-yield” and, therefore, has less stringent groundwater remediation standards.
- The concentration and size of the plume will have been reduced to a point that growth or migration of the remaining plume will be controlled by natural attenuation.

Information is not provided about the analytical methods used in this application, however no exceptions to established quality assurance/quality control (QA/QC) protocols were noted.

Technology Cost [3]

Capital costs for construction of the extraction/injection trenches and control building were approximately \$600,000. Annual costs for operation, maintenance and monitoring are approximately \$100,000.

Summary Observations and Lessons Learned [1,3]

Methanol addition was found to increase the rate of biodegradation of TCE at this site, based on the reduction of TCE concentration and increase in the ratio of cis-1,2-DCE to TCE. This site is planning to stop using active bioremediation after four years of system operation (three years of methanol addition) to allow use of natural attenuation. According to the site contractor, natural attenuation will be used to prevent future migration of the plume, and to achieve stable or declining contaminant concentrations.

Excessive biomass formation, leading to a reduced flow rate, was found to be a concern for addition of methanol. Excess biomass was not noted during the period when nutrients alone were added; however, a significant increase in biomass formation was noted after addition of methanol. To remedy this, the site contractor modified their methanol addition to a batch system.

The site contractor found that it was difficult to balance the system hydraulics between the extraction and infiltration trenches, and that it required approximately one year of operating time to achieve a balance.

In addition, they found it difficult to interpret the treatment performance data because of the non-homogeneous nature of the initial groundwater quality, and dilution due to recharge of rainwater and clean water from beyond the planned treatment area.

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Case Study #5
Pilot and Full Scale Molasses Injection at the Avco Lycoming Superfund Site,
Williamsport, Pennsylvania

Summary Information [1-6]

Site Name, Location	Avco Lycoming Superfund Site, Williamsport, Pennsylvania
EPA ID Number	PAD003053709
Mechanism(s)	Anaerobic Reductive Dechlorination (Cometabolic and Direct)
Technology	Electron Donor Addition (Molasses)
Configuration	Direct Injection
Technology Scale	Pilot and Full
Media/Matrix Treated	Groundwater
Contaminants Targeted	TCE, DCE, VC, hexavalent chromium, cadmium
Period of Operation	Pilot study October 1995 to March 1996; Full-scale system ongoing, data available from January 1997 to July 1998

Site History/Source of Contamination [1,6,7,8]

The Avco Lycoming Superfund site (Lycoming) is a 28-acre facility located in Williamsport, Pennsylvania. Since 1929, various manufacturing companies have operated at the site, including a bicycle and sewing machine plant, a sandpaper plant, a tool and die shop, a silk plant, and an aircraft engine plant, that is currently operating at the site. Past waste handling practices, including disposal of wastes in a dry well and coolant well, spillage and dumping of wastes from metal plating areas, and storing sludge in a holding lagoon, resulted in soil and groundwater contamination at the site.

In the mid-1980's, the state identified the Lycoming site as the source of volatile organic compound (VOC) contamination in the local municipal water authority well field located 3,000 ft south of the site. Contaminants include trichloroethene (TCE) and trans-1,2-dichloroethene (DCE), and metals, including hexavalent chromium and cadmium. A pump and treat system was installed at the site in the late 1980's to remediate on-site and off-site groundwater contamination. The Lycoming site was placed on the National Priority List on February 21, 1990, and a Record of Decision (ROD) was issued for this site in June 1991 requiring pump and treat for the shallow groundwater beneath the facility property, followed by discharge to a nearby stream. Design of this system was suspended pending resolution of a permit for the discharge.

In May 1995, the potentially responsible party (PRP) proposed an alternative for remediating the shallow groundwater that involved using an *in situ* bioremediation system that included molasses injection and air sparging/soil vapor extraction (SVE). Pilot studies were conducted from October 1995 to June 1996. A new ROD was issued in December 1996 replacing the pump and treat with an *in situ* remedy. A full-scale molasses injection system was installed and has been operating at the site since January 1997.

Although the air sparging/SVE system was pilot tested, and construction of a full-scale system begun in October 1997, construction was suspended in the Spring of 1998, due to higher than anticipated water levels at the site. The PRP is planning to submit a new proposal to EPA for remediation of the organic-contaminated groundwater at the site. Therefore, this case study focuses on the molasses injection technology.

Geology/Hydrogeology/Contaminant Characterization [1,3]

Site geology consists of a sandy silt overburden overlying a fractured bedrock and a fractured limestone. The target area for the *in situ* treatment is the shallow overburden to approximately 25 ft below ground surface (bgs), and covers approximately two acres. The maximum concentrations measured in this area in late 1996 were TCE, 0.7 mg/L, hexavalent chromium, 3 mg/L, and cadmium, 0.8 mg/L.

Matrix Characteristics at Lycoming Superfund Site [1,3,8]

Parameter	Value
Soil Type	Sandy silt
Depth to Groundwater	10 to 15 ft bgs
Thickness of Aquifer	10 to 12 ft
Fraction of Organic Carbon	Not available
DNAPL Present	Not identified
Hydraulic Conductivity	0.2 to 24 ft/day
Groundwater Velocity	0.02 to 2.3 ft/day

Technology Description [1,7,8]

Pilot Study. The molasses pilot study was conducted from November 1995 to June 1996. In the pilot study, an *in situ* reactive zone was created to reduce groundwater concentrations of hexavalent chromium and cadmium. Monitoring data showed that chromium concentrations were reduced from 7 mg/L to less than 0.05 mg/L in the test zone, and that the technology also created conditions needed to reductively dechlorinate the CAHs. For example, during the pilot study, redox conditions were shown to be strongly reducing, at less than -300mV. The air sparging/SVE pilot system took place from October 1995 to May 1996.

Full-scale System. The full-scale molasses injection system, a proprietary technology owned by ARCADIS Geraghty & Miller, was constructed in late 1996 and began operating in January 1997. As shown in Figure 1, the molasses injection system consists of 20 four-inch diameter injection wells, ranging in depth from 19 to 30 ft, completed in the overburden. Each well is connected to a 10 ft square treatment building by 3/4-inch diameter piping. Molasses is added two times each day at variable concentrations and rates based on the results from system monitoring. Figure 2 shows the monitoring wells network at the site. A programmable logic controller monitors and controls the feed rate and frequency of substrate addition. Table 1 summarizes the operating parameters for the system.

Figure 1: Molasses Injection System Used at Lycoming Superfund Site [2]

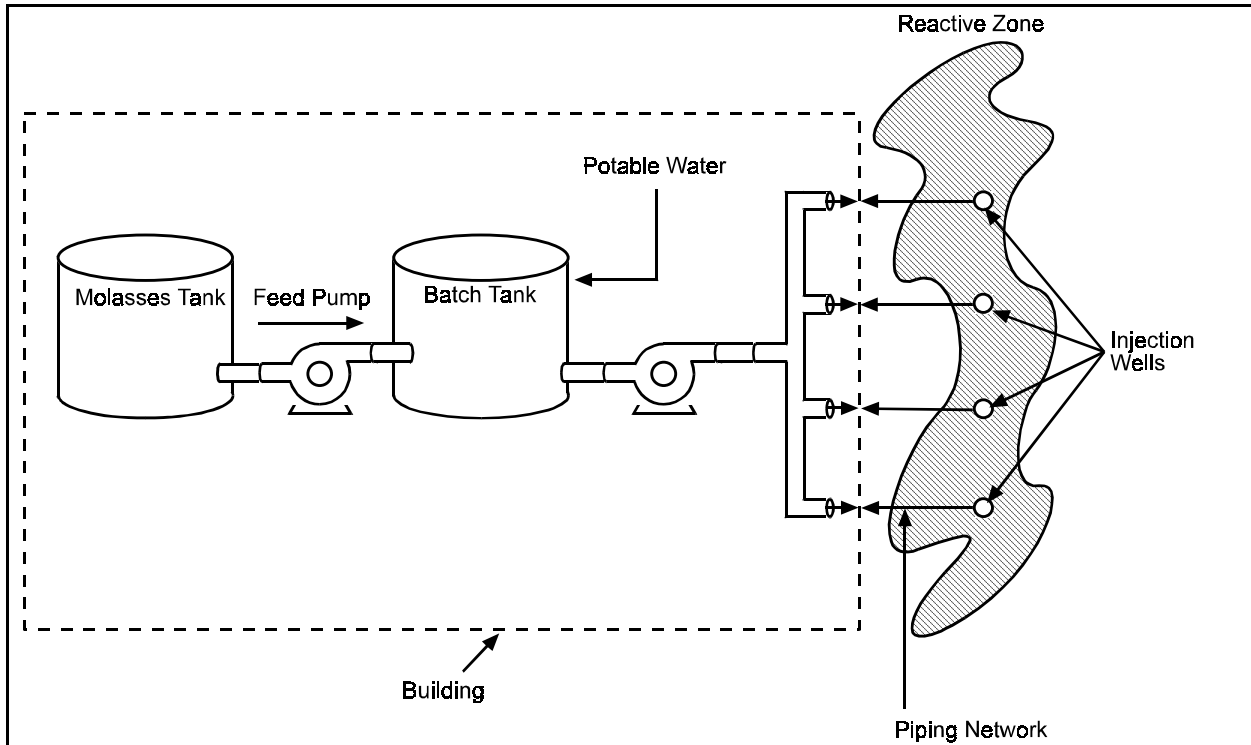


Figure 2: Monitoring Well Network at the Site [7]

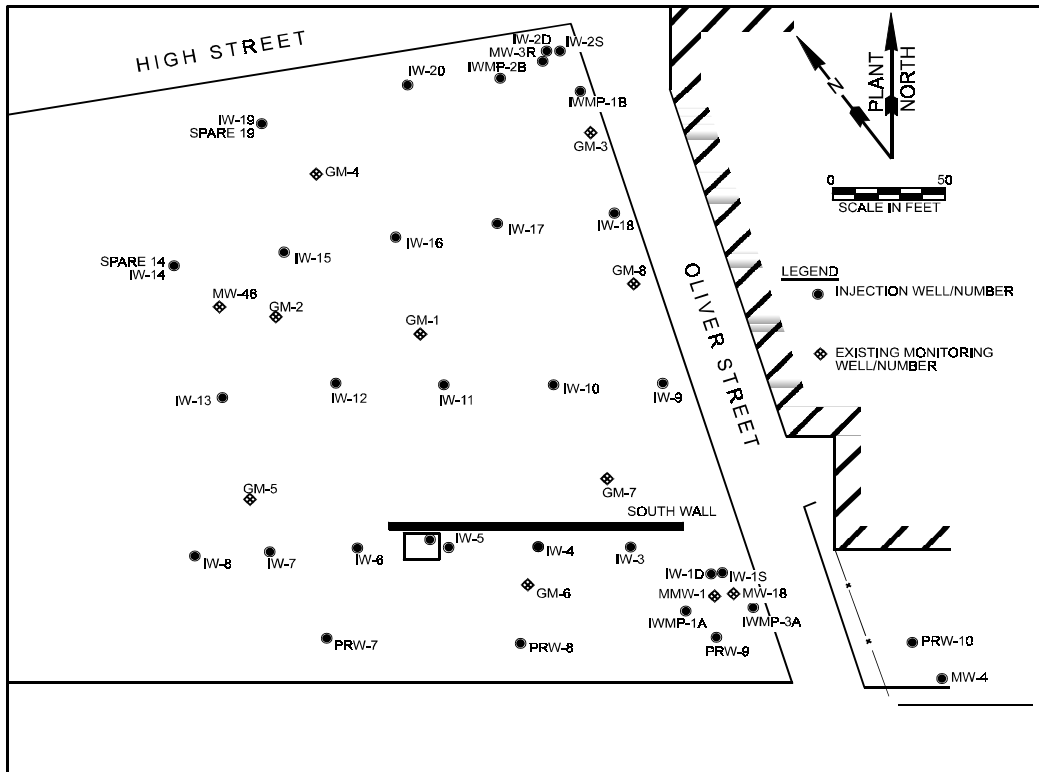


Table 1. Operating Parameters Used at Lycoming Superfund Site [1]

Operating Parameter	Value
Substrate (molasses) addition	Not provided
Redox potential	< -300mV

Technology Performance [7,8]

The 1996 ROD specified the following cleanup goals for groundwater: TCE (5 ug/L), 1,2-DCE (70 ug/L), VC (2 ug/L), cadmium (3 ug/L), hexavalent chromium (32 ug/L), and manganese (50 ug/L).

Groundwater monitoring data are available for January 1997 to July 1998. Samples were collected from 16 wells, (GM-1 through GM-8; MW-3R, 4, 18, and 46; and PRW-7, 8, 9, and 10), as shown on Figure 1. Figures 3 and 4 show the geochemical conditions of the groundwater in January 1997 and July 1998, respectively, using the following indicator parameters - redox potential, sulfide, and total organic carbon (TOC). The January 1997 results, collected prior to initiation of molasses injections, were used as the baseline conditions. The data showed that anaerobic and reducing conditions were present only near two of the site monitoring wells (GM-3 and MW-18) located near the northeastern and southeastern corners of the site.

Figure 3: Distribution of Groundwater Indicator Parameters, Baseline Conditions, January 1997 [2,8]

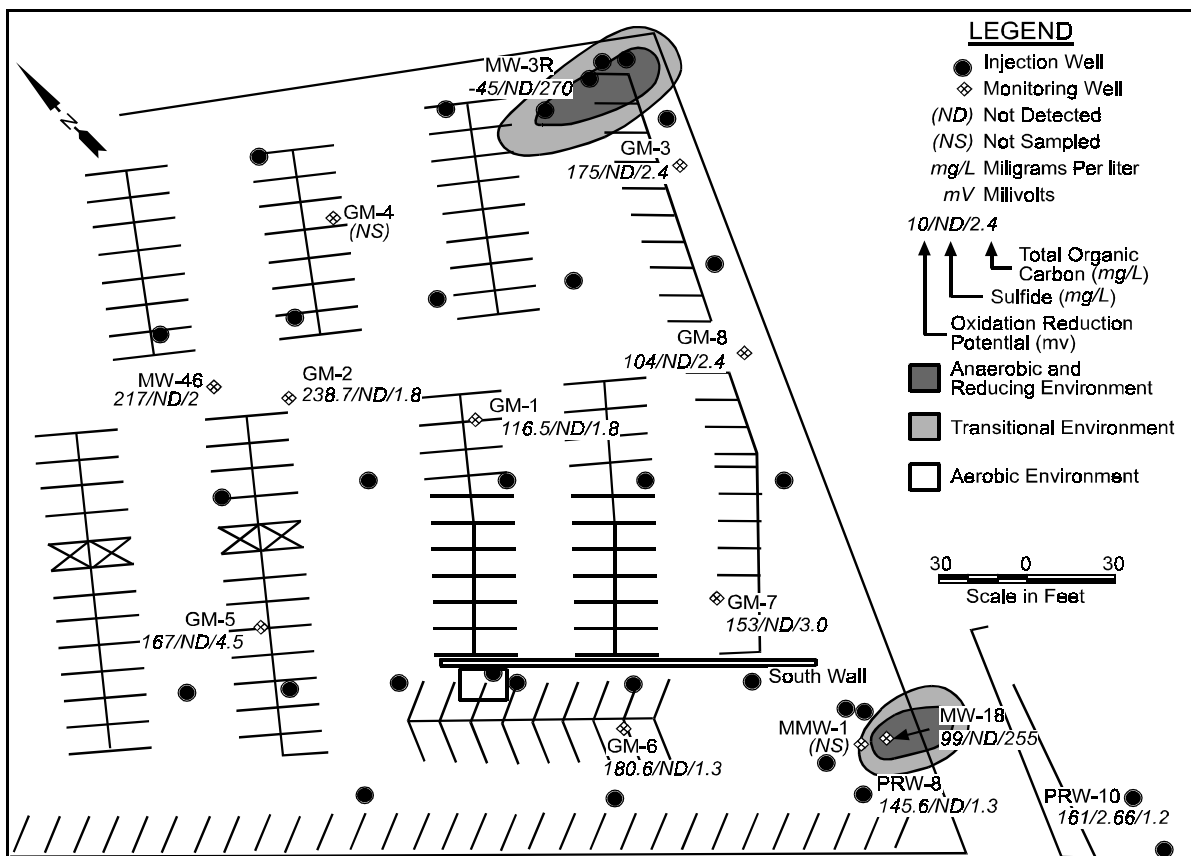
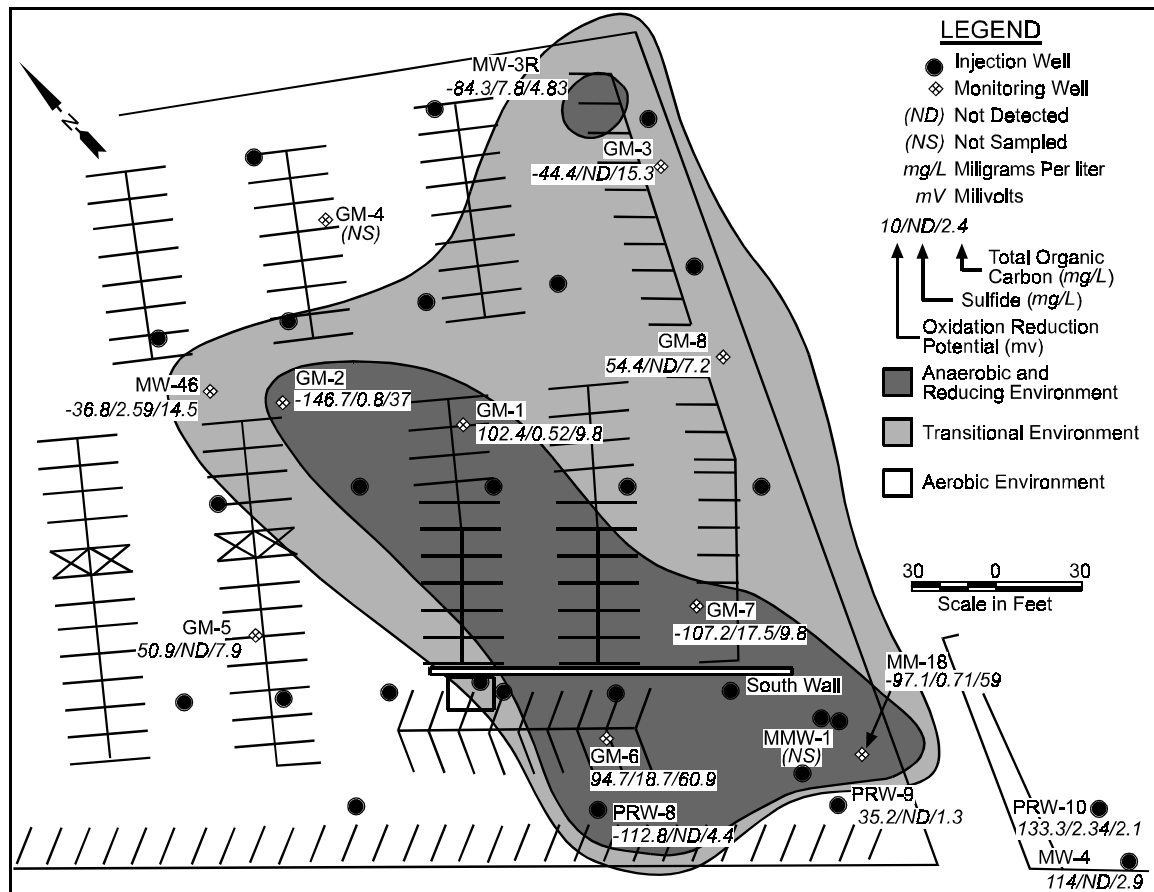


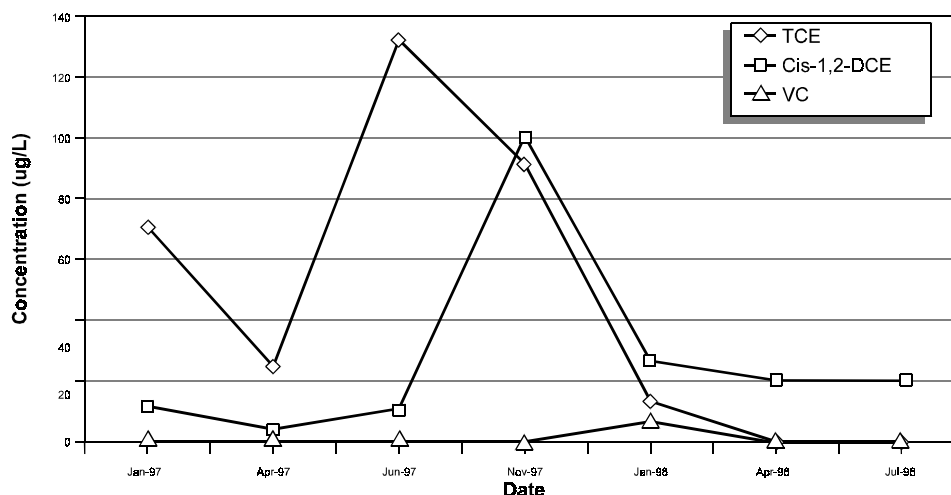
Figure 4: Distribution of Groundwater Indicator Parameters, Reactive Zone Established, July 1998 [8]



Data collected in July 1998 (Figure 4) show that the redox levels have decreased to anaerobic conditions in many of the wells that had previously indicated an aerobic environment, indicating that anaerobic and reducing conditions have been expanded to include a majority of the eastern portion of the treatment area. The presence of sulfide, the reduced product of sulfate, indicated that conditions were sufficient to promote reductive dechlorination of chlorinated aliphatic hydrocarbons. Concentrations of TOC also were increased in the treatment area. (DO data collected during the study were not collected using a flow-through cell. The PRP contractor reported that these data were believed to be significantly biased high, however, no additional information was provided).

As of July 1998, concentrations of TCE, DCE, and hexavalent chromium have been reduced to below than their cleanup goals in many of the monitoring wells at the site. Concentrations of hexavalent chromium been reduced by more than 99% from 1,950 ug/L to 10 ug/L. Figure 5 presents the results of analyses for TCE, DCE, and VC between January 1997 and July 1998 for monitoring well GM-7, which is located near the South Wall and within an area that was converted from aerobic to anaerobic during the first 18 months of treatment. As shown on Figure 5, the concentration of TCE was reduced by 90% from 67 ug/L to 6.7 ug/L. The concentration of DCE initially increased from 7 ug/L to 100 ug/L after 10 months of treatment, indicating the successful dechlorination of TCE, then decreased to 19 ug/L by July 1998. Similarly, the initial concentration of VC increased from below the detection limit of <1 ug/L to 5 ug/L after 10 months of treatment, then decreased to below the detection limit by July 1998.

**Figure 5: Analytical Results for Well GM-7 at Lycoming Superfund Site [8]
(January 1997 to July 1998)**



While specific information about the analytical methods or data quality were not provided in the available references, no exceptions to the quality assurance/quality control (QA/QC) protocols were noted.

Technology Cost [3,8]

ARCADIS Geraghty & Miller, Inc. reported project costs for the full-scale molasses injection system at the Lycoming site to be about \$220,000 for construction and about \$50,000 per year for operation and maintenance. The cost for the pilot study at this site, including preparation of a work plan, was about \$145,000.

Summary Observations and Lessons Learned [1,3]

The use of molasses injection was shown to create an anaerobic reactive zone within an 18-month period, with concentrations of TCE, DCE, and hexavalent chromium reduced to below the cleanup goals in many of the wells.

This was one of the earliest full-scale applications of this technology at a Superfund site. According to ARCADIS Geraghty & Miller, this technology was shown to save substantial resources when compared to pump and treat.

The pilot study demonstrated the ability of the technology to create strongly reducing redox conditions and to reduce concentrations of chlorinated aliphatic hydrocarbons and hexavalent chromium. The results of the pilot study were used in the design and operation of the technology on a full-scale basis at the site.

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Case Study #6
Pilot and Full Scale Anaerobic In-situ Reactive Zone at an Abandoned Manufacturing Facility, Emeryville, California

Summary Information

Site Name, Location	Abandoned Manufacturing Facility, Emeryville, California
EPA ID Number	Not Applicable
Mechanism(s)	Anaerobic Reductive Dechlorination and Metal Precipitation
Technology	Electron Donor Addition (Molasses)
Configuration	Direct Injection
Technology Scale	Pilot and Full
Media/Matrix Treated	Groundwater
Contaminants Targeted	TCE, hexavalent chromium
Period of Operation	Pilot study – August 1995 to February 1996 Full scale system-ongoing, data available from April 1997 to October 1998

Site History/Source of Contamination [1]

From 1952 until 1995, metal plating operations, including nickel plating, were performed at a manufacturing facility located in Emeryville, California (actual site name confidential). Solvents were used in degreasing operations until 1992, when they were replaced with a liquid-alkaline soak process. Plating operations were discontinued in 1995, and the associated plating equipment has subsequently been removed from the site. Operations at the site resulted in the groundwater becoming contaminated with chlorinated solvents and metals.

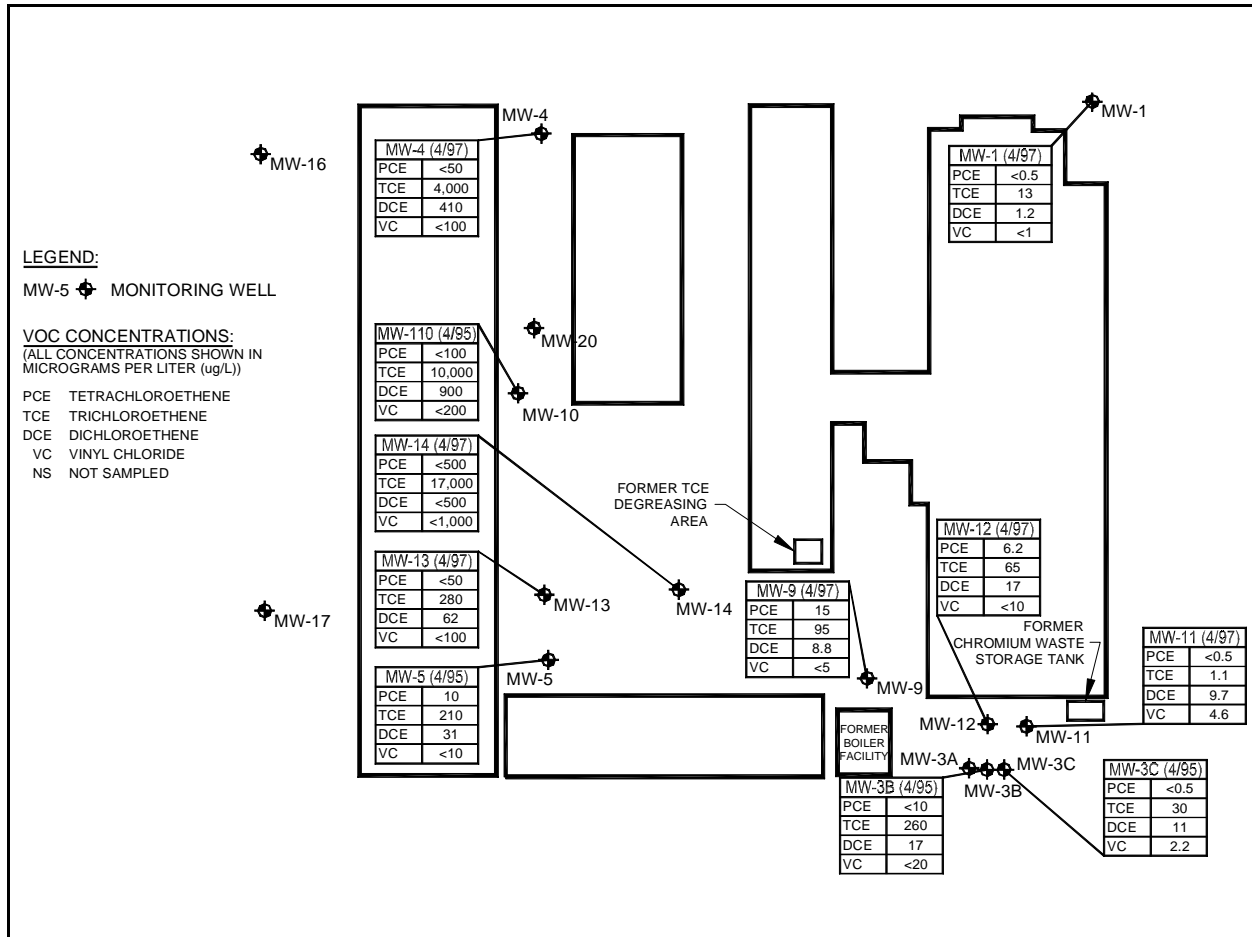
Between 1977 and 1985, 24 groundwater monitoring wells were installed at the site and on adjacent properties. Figure 1 shows the location of the 14 on-site monitoring wells. Elevated levels of chromium and trichloroethene (TCE) was detected in groundwater in the late 1970s and early 1980s. The cleanup of the site is being completed under a state voluntary cleanup program. In 1995, the site owner initiated a pilot study to evaluate anaerobic reductive dechlorination and metals precipitation via an in-situ reactive zone as a possible remedy for the site (as a potential alternative to a conventional pump and system). The following case study primarily focuses on the reductive dechlorination of TCE; limited data on the precipitation of hexavalent chromium was provided in the available references.

Geology/Hydrogeology/Contaminant Characteristics [1]

The geology of the site geology consists of interbedded sand and clay units. Groundwater is found at depths of 3.5 to 8 feet below ground surface (bgs). Groundwater velocity is estimated to be 60 feet per year.

TCE and chromium are the primary contaminants in the groundwater at the site. TCE concentrations from April 1995 (prior to initiation of the pilot study) were as high as 17,000 ug/L (Well MW-14). Historical groundwater data from on-site wells indicated that, over the past 10 years, TCE concentrations have been slowly decreasing. For example, TCE concentrations in Well MW-10 were 12,000 µg/L during a June 1985 sampling event and 10,000 µg/L during an April 1995 sampling event.

Figure 1: Pre-Injection CAH Concentrations, Abandoned Manufacturing Facility, Emeryville, California (April 1995) [1]



Matrix Characteristics at Abandoned Manufacturing Building [1]

Parameter	Value
Soil Type	Interbedded sand and clay units
Depth to Groundwater	Approximately 3.5 to 8 feet
Thickness of Aquifer	Not available
Fraction of Organic Carbon	Not available
DNAPL Present	None identified
Hydraulic Conductivity	Not available
Groundwater Velocity	60 feet per year

Technology Description [1,3]

Pilot Study

The pilot study was conducted between August 1995 and February 1996. The pilot study was performed to determine if the rate of TCE degradation and metals precipitation could be enhanced by an anaerobic in-situ reactive zone. Groundwater monitoring data, collected prior to the start of the pilot study, indicated that limited reductive dechlorination of TCE to cis-1,2-dichloroethene (DCE) was occurring, but that the rate of dechlorination was limited due to the biogeochemical conditions at the site (the organic carbon source was depleted or the environment was not sufficiently reducing). Vinyl chloride (VC), the degradation product of cis-1,2-DCE, was either not detected or was sporadically detected in many of the wells. According to the site contractor, DCE and VC may have been present in some wells prior to start of the pilot study, but were not detected because of high method detection limits (e.g., 1,000 $\mu\text{g/L}$ for DCE and 2,000 $\mu\text{g/L}$ for VC).

To establish the anaerobic reactive zone, a mixture of molasses, biologically inoculated solution (supernatant), and tap water was injected into the subsurface. Injection of the supernatant was needed because of low plate counts observed in one well during a baseline sampling event. The supernatant used for the pilot study was from the anaerobic treatment system of a local municipal authority.

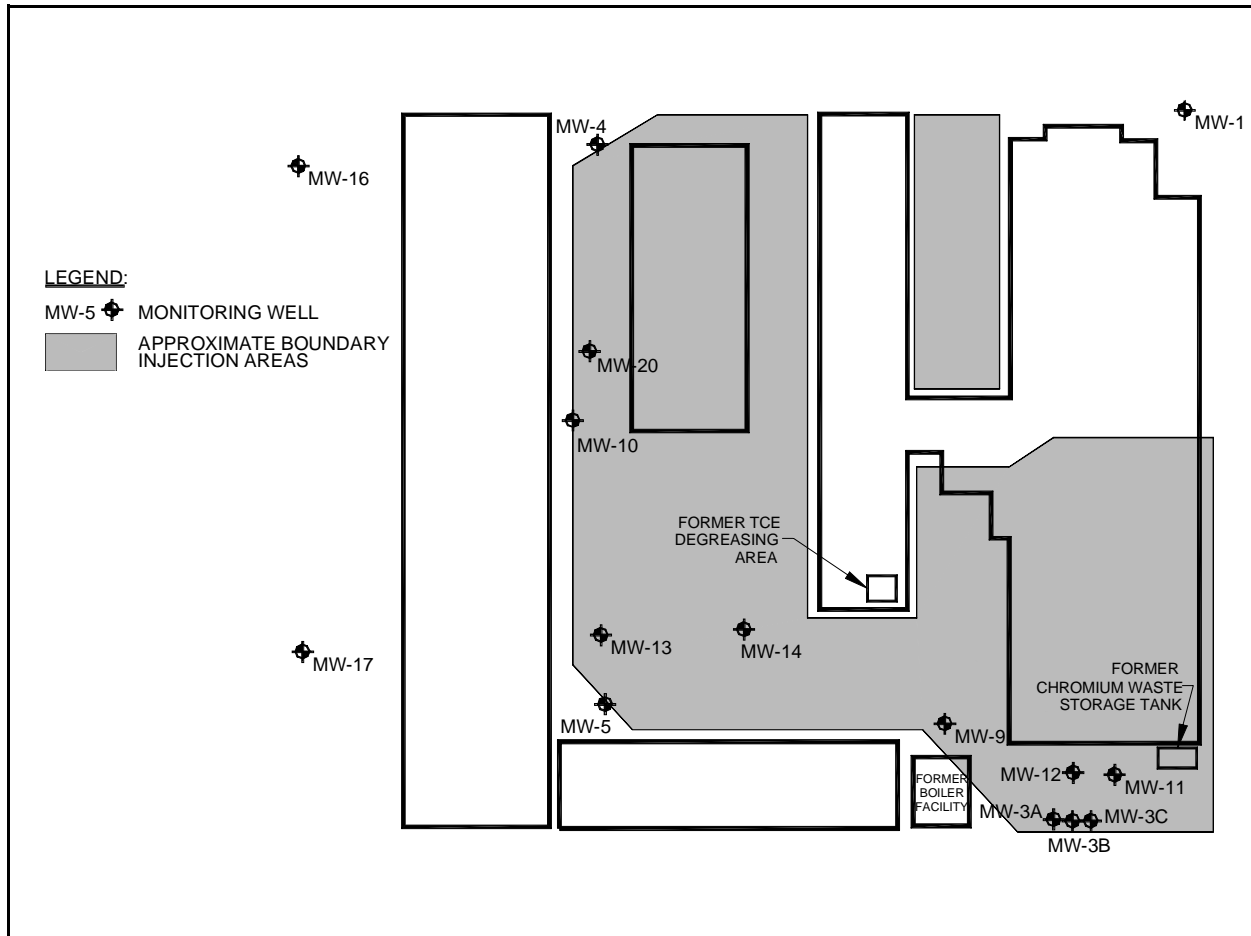
The results of the pilot study indicated that the historical reductive dechlorination rate at the site could be enhanced via the injection of the molasses solution. For example, TCE concentrations in Well MW-10 were reduced from 10,000 $\mu\text{g/L}$ in April 1995 to 4,200 $\mu\text{g/L}$ in February 1996.

Full-Scale System

In April 1997, ninety-one temporary injection points were installed at the site, as shown in Figure 2. The injection points are located in two areas due to the location of existing buildings. Each injection points was installed to a depth of 24 feet bgs.

The full-scale system has been operating since April 1997 and data are available through October 1998. Two molasses injection events have been performed at the site, in April 1997 and in February 1998. Each molasses injection event included a mixture of water, molasses, and a small amount of supernatant (to provide additional bacteria capable of degrading TCE). During the first injection event, each injection point received 25 gallons of molasses, 1 gallon of supernatant, and 125 gallons of water. Information about the volume and composition of the solution used in the second injection event was not available. The reagent was mixed on-site and manually injected into the subsurface using a centrifugal pump.

Figure 2: Full-Scale Injection Area, Abandoned Manufacturing Facility, Emeryville, California [2]



Technology Performance [1,2]

Performance data are available through October 1998. Figure 3 presents the data on concentrations of PCE, TCE, DCE, and VC in on-site wells as of October 1998. Figures 4 and 5 show the change in concentrations of TCE, DCE, and VC from December 1996 through October 1998 for Wells MW-4 and MW-14, respectively. Well MW-14 is located in the source area and MW-4 is in the mid-plume area. Figure 6 shows the average TCE, DCE and VC concentrations in the on-site monitoring wells within the remediation area (MW-4, MW-10, W-13, and MW-14).

As shown in Figure 3, the maximum contaminant concentrations measured in groundwater at the site as of October 1998 were PCE (0.75 ug/L), TCE (17 ug/L), DCE (1,400 ug/L), and VC (180 ug/L). Figures 4 and 5 show that TCE, DCE, and VC concentrations in wells MW-14 and MW-4 were reduced to below the detectable levels by October 1998. Initial DCE and VC concentrations increased following the first reagent injection, but then declined by October 1998. According to the site contractor, the trends for TCE degradation products (DCE and VC) indicate that TCE is being reductively dechlorinated to ethene. As shown in Figure 6, concentrations of TCE in wells located within the remediation area have decreased by 99% (3,040 µg/L in April 1995 to 4 µg/L in October 1998).

Figure 3: CAH Concentrations - October 1998, Abandoned Manufacturing Facility, Emeryville, California [2]

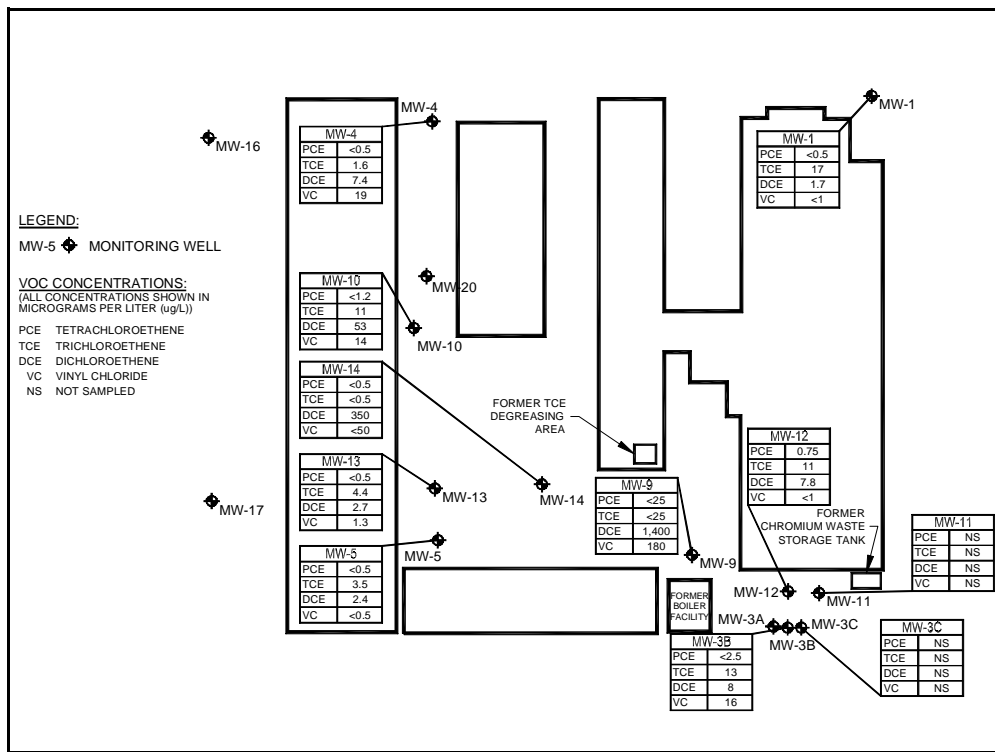


Figure 4: Analytical Results for Well MW-4, Abandoned Manufacturing Facility, Emeryville, California [2]

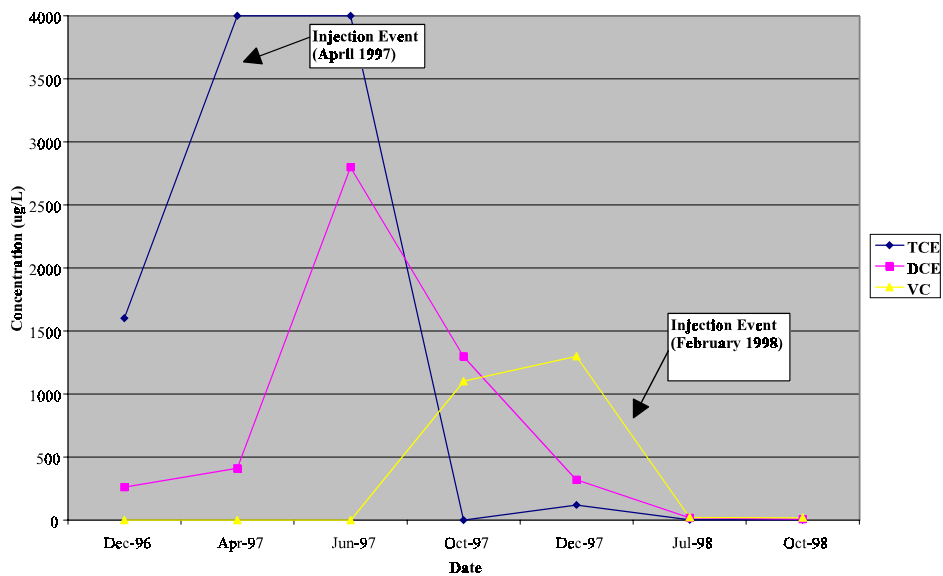


Figure 5: Analytical Results for Well MW-14, Abandoned Manufacturing Facility, Emeryville, California [2]

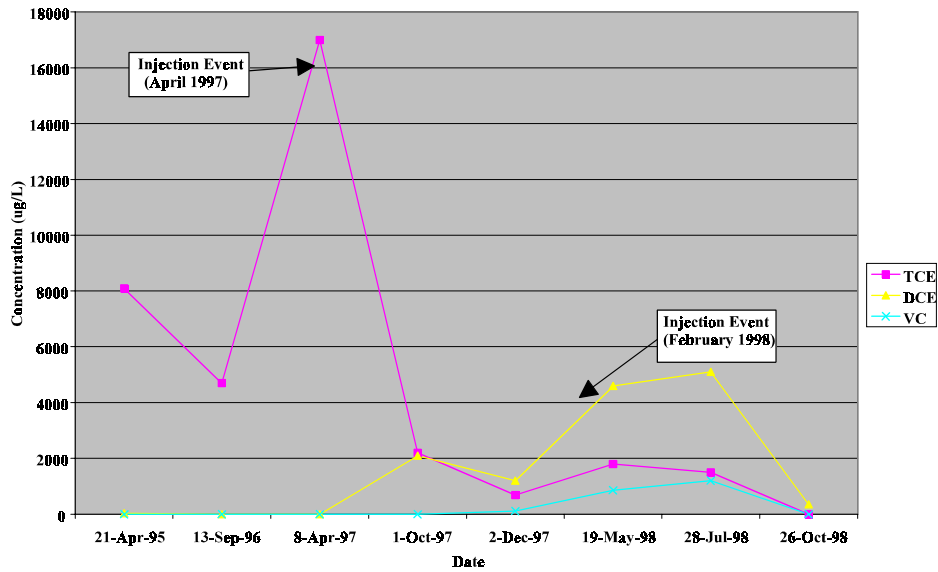
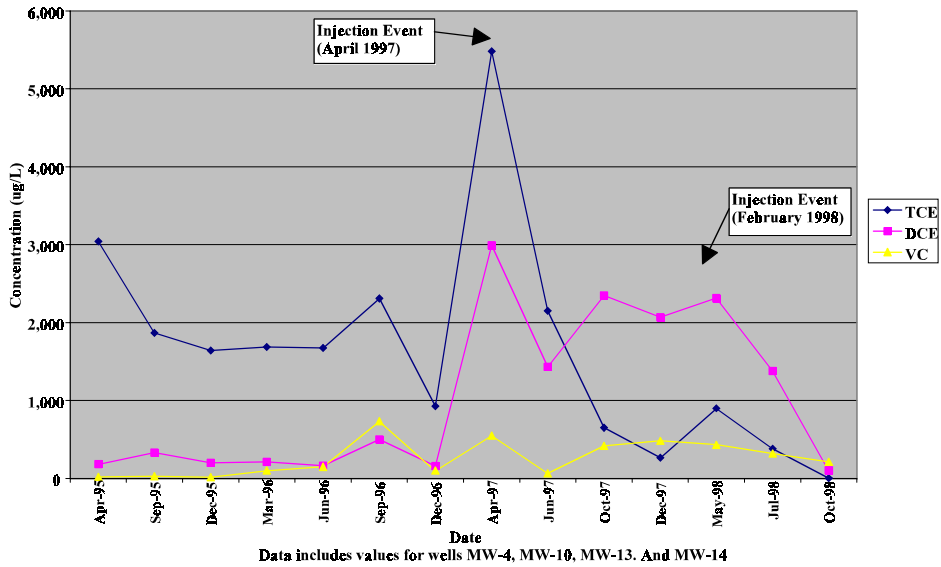


Figure 6: Average Concentrations, On-Site Wells in Remediation Area, Abandoned Manufacturing Facility, Emeryville, California [2]



In addition, the average concentrations of total chromium and hexavalent chromium in the injection area have been reduced by approximately 98% and 99%, respectively, and some of the wells where historic hexavalent chromium concentrations were in excess of 100,000 $\mu\text{g/L}$ are now less than the detection limit (5 $\mu\text{g/L}$).

Technology Costs [1]

The overall project cost is approximately \$400,000. No further information was provided about the components of this cost, such as a breakdown of capital or operations and maintenance (O&M) costs.

Summary Observation and Lessons Learned [1,2,3]

The injection of molasses reagent solution created conditions favorable for the reduction in TCE, DCE, VC, and chromium concentrations in the subsurface. During an 18-month period of full-scale operation, average concentrations of TCE were reduced by 99%, from more than 3,000 ug/L to 4 ug/L . Average concentrations of hexavalent chromium were reduced by 99% to below detection levels.

The solution of molasses, supernatant, and water was injected through 91 temporary injection points installed using a Geoprobe™. According to the remediation contractor, the use of a Geoprobe™ allowed the injection points to be installed relatively quickly and at low cost.

A pilot study was conducted prior to the full-scale operation. The pilot study showed that the rate of reductive dechlorination could be enhanced with the use of an injected molasses solution.

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Case Study # 7
Sequential Anaerobic/Aerobic Biodegradation of PCE Field Demonstration
at Watertown, Massachusetts

Summary Information [1,3]

Site Name, Location	Watertown, Massachusetts	
EPA ID Number	Not provided	
Mechanism(s)	Anaerobic Reductive Dechlorination (Cometabolic and Direct)	Aerobic Oxidation (Cometabolic and Direct)
Technology	Nutrient Addition Electron Donor Addition (Lactate)	Electron Acceptor Addition (Oxygen) Electron Donor Addition (Propane)
Configuration	Groundwater Recirculation	
Technology Scale	Field Demonstration	
Media/Matrix Treated	Groundwater	
Contaminants Targeted	TCE, PCE	
Period of Operation	Anaerobic: November 1996 to July 1997; Aerobic: August 1997 to ongoing (data available through October 1997)	

Site History/Source of Contamination [1,2,5]

The Watertown site has been used since the late 1800's for a variety of operations, including a coal gas manufacturing plant, which ceased operations in the 1930's, and a metal plating shop, which ceased operations in 1990. The site is currently being used as a manufacturing facility for electric switch assembly. Soil and groundwater at the site are contaminated with chlorinated solvents, including trichloroethene (TCE) and tetrachloroethene (PCE), from past operations and waste disposal practices.

A field demonstration of the Two-Zone Plume-Interception Treatment Technology developed by Harding Lawson Associates (HLA, formerly ABB Environmental Services, Inc.) was conducted at the Watertown site under the Superfund Innovative Technology Evaluation (SITE) program. The field demonstration is currently ongoing. This report addresses the results through October 1997.

Geology/Hydrogeology [1,2]

Soil at the Watertown site consists of about 13 feet (ft) of sand and gravel over approximately 7 ft of silty sand. Glacial till about 5-ft thick acts as an aquitard and separates the sand layer from the underlying bedrock (light grey, moderately weathered, amorphous Cambridge Argillite). Groundwater is encountered approximately 8 ft below ground surface (bgs).

Matrix Characteristics for the Watertown Site [1]

Matrix Characteristic	Value
Soil Type	Sand, gravel, and silt
Soil Permeability	Not Provided
Depth to Groundwater	8 ft bgs
Fraction of Organic Carbon	Not Provided
DNAPL Present	None Identified
Hydraulic Conductivity	Not Provided
pH	Not Provided
Porosity	Not Provided
Hydraulic Gradient	Not Provided
Groundwater Velocity	Not Provided

Technology Description [1,2]

The technology demonstrated at the Watertown site is a “two-zone” enhanced bioremediation process that uses sequential anaerobic and aerobic biodegradation processes to degrade PCE and TCE. The first zone is designed to operate under highly reducing conditions to stimulate anaerobic bacteria to dechlorinate solvents. While complete degradation can occur under these conditions, according to the vendor, the process may be slow; whereas under aerobic conditions, completing the degradation of DCE and VC is relatively fast. Therefore, a second zone, designed to operate under aerobic conditions, is used to stimulate methanotrophic bacteria to complete degradation by oxidizing DCE and VC.

The field demonstration system, shown in Figure 1, consisted of three injection wells and three extraction wells. The injection/extraction wells were used to develop a groundwater recirculating cell that covered a surface area of approximately 10 ft by 20 ft. The wells were constructed of 4-inch diameter PVC pipe and screened from 13 to 20 ft bgs. Five monitoring wells, with 5-ft screens at 15 to 20 ft bgs, were located between the injection and extraction wells, and additional monitoring wells were located outside of the treatment cell, as shown in Figure 2. Nutrients and a carbon source were injected into the groundwater through the three “upgradient” wells and extracted through the three “downgradient” wells.

During the period of operation, a relatively constant recirculating flow rate of 0.25 gallons per minute (gpm) was used along with an amendment injection rate of 10 mL/min or about four gallons per day (approximately 1% of the recirculating flow). The system was operated under anaerobic conditions for eight months (through late July 1997), then changed to aerobic conditions, as described below.

Figure 1: Process Diagram [1]

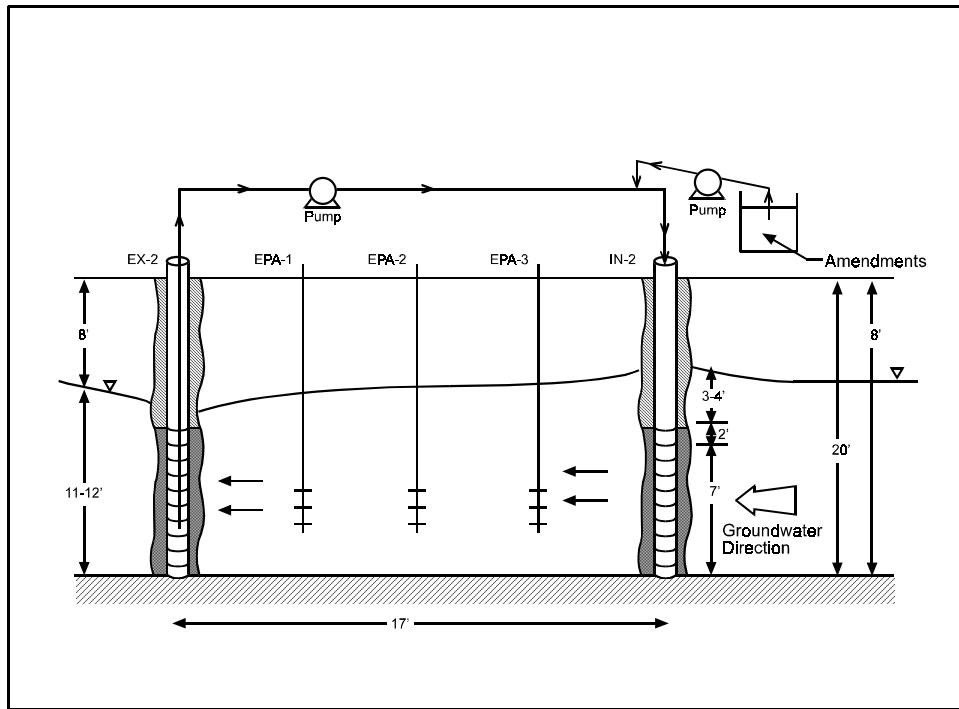
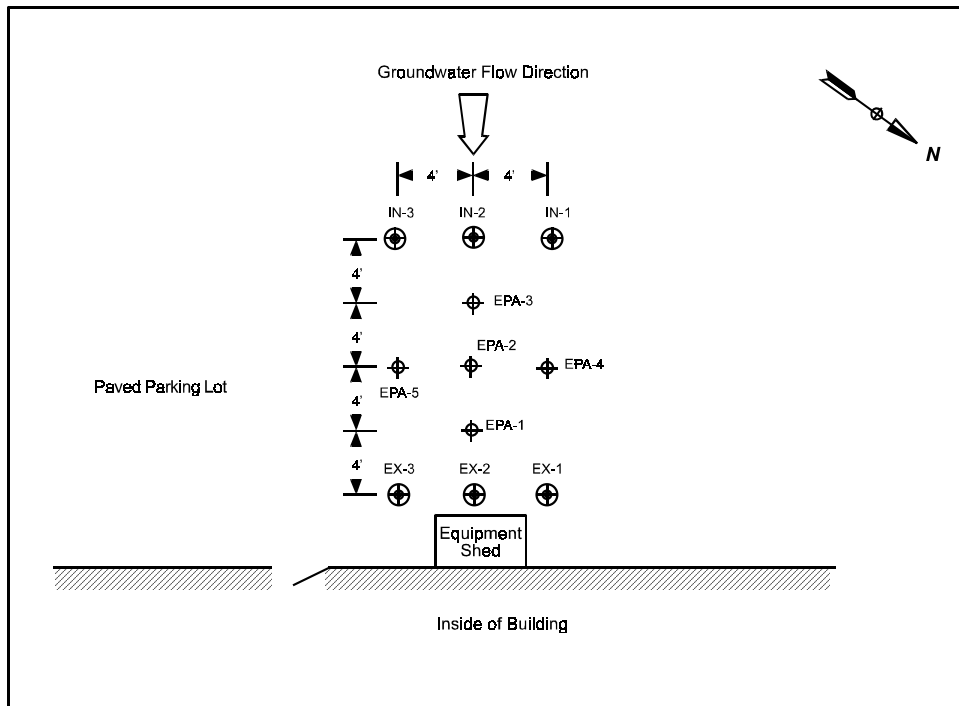


Figure 2: Well Locations [1]



Anaerobic conditions (November 1996 to July 1997):

Amendments were added to the recirculating flow (continuous addition) to enhance anaerobic biological dechlorination. Initially, ammonia chloride (25 mg/L), potassium tripolyphosphate (25 mg/L), lactic acid (100 mg/L), yeast extract (5 mg/L), and sodium hydroxide (at concentrations needed to neutralize the pH of the amendment batch) were added. From April to June 1997, amendments were pulsed into the system and the concentration of lactic acid was increased to 250 mg/L to lower redox conditions. In June 1997, the concentration of lactic acid was increased to 350 mg/L and the system was operated under anaerobic conditions until late July 1997.

Aerobic conditions (August 1997 to ongoing - data available through October 1997):

In July 1997, “socks” containing oxygen release compound (ORC) were suspended inside the three injection wells to provide a continuous release of oxygen into the recirculating groundwater flow. However, it took about a month before aerobic conditions were established. The reason for the lag period was attributed to the presence of residual carbon in the system that had to be degraded before aerobic conditions were established in the injection wells.

Because levels of methane in the groundwater had decreased from 150 to 200 ug/L at the beginning of the demonstration to 50 to 100 ug/L at the start of the aerobic phase, methane was added to the system to enhance aerobic bacteria activity. Methane was added on a weekly basis starting about two months after the ORC socks were installed in the injection wells.

Technology Performance [1]

Anaerobic Conditions (November 1996 to July 1997):

During the first four months of operation under anaerobic conditions (November 1996 to February 1997), limited degradation was observed. Data from February 11, 1997 showed that the concentrations and relative ratio of PCE and TCE to DCE and VC were relatively uniform throughout the treatment cell.

After four to five months of operation (March to April 1997), significant increases in DCE were observed along with decreases in TCE concentrations, indicating that reductive dechlorination was occurring. However, no significant increases in VC concentrations were observed until July 1997, 8 months after operations began.

Methane levels declined continuously during this period from 0.2 to 0.3 mg/L at the beginning of the demonstration to 0.05 to 0.1 mg/L in July, 1997. These results indicate that methanotrophic conditions were not achieved during the anaerobic phase; most of the reductive dechlorination was therefore attributed to sulfate-reducing bacteria. Sulfide was detected in wells during the last two months of the anaerobic phase.

By July 1997, TCE concentrations had been reduced from about 12 mg/L at the beginning of the demonstration to less than 1 mg/L. In addition, there was an overall reduction of about 80% in the mass of total VOCs, as measured in well IN-2 as of July 1997.

Aerobic Conditions (August 1997 to ongoing - data available through November 1997):

Data on the aerobic phase of the demonstration have been collected through November 1997. These data show that VOC levels, primarily DCE and vinyl chloride, have started to decrease in the groundwater. In addition, DCE epoxide, a transient biodegradation product of aerobic degradation of DCE, was detected during two sampling events, indicating that aerobic VOC-degrading bacteria have been stimulated. HLA is continuing to collect data on the aerobic phase of the demonstration. During the aerobic phase over a period of about 3 months, the degradation half-life for vinyl chloride was approximately 45 days, and for the latter month of this period was about 22 days.

Technology Cost [4]

The cost for the field-scale pilot study through November 5, 1997 was approximately \$150,000. No estimates were provided about the projected costs for a full-scale system using this technology.

Summary Observations and Lessons Learned [1,2,4]

Under anaerobic conditions, TCE in groundwater was reduced by reductive dechlorination (from 12 mg/L to less than 1 mg/L) and there was an overall reduction of about 80% of the total VOC mass in well IN-2. Data indicate that methanogenic conditions were not achieved during the anaerobic phase and most of the reductive dechlorination was attributed to sulfate-reducing bacteria.

During the anaerobic phase, there was a lag time of four to five months before significant reductive dechlorination was observed; substantial increases in vinyl chloride were observed, eight months after the demonstration began. Possible reasons for the lag time include: the redox conditions may have been too high; there may have been insufficient electron donor present (lactic acid); or an acclimation period may have been required before anaerobic degradation occurred.

A period of about one month was required to establish aerobic conditions after ORC socks were placed in the wells. This lag time was attributed to the presence of residual carbon that had to be degraded before aerobic conditions could be established. Initial results indicate that VOC levels, primarily DCE and vinyl chloride, are decreasing. However, HLA is continuing collect data on the aerobic phase.

The EPA contact for this application stated that future applications should consider not starting in the winter, start when the anaerobic process can go quickly, use a higher level of lactate, and drive the oxidation potential down quickly. [4]

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Case Study # 8
Bioaugmentation (Accelerated Anaerobic Bioremediation) Field Demonstration
at Area 6 of the Dover Air Force Base, Dover Delaware

Summary Information [1]

Site Name, Location	Dover Air Force Base, Area 6, Dover, Delaware
EPA ID Number	DE8570024010
Mechanism(s)	Anaerobic Reductive Dechlorination (Cometabolic and Direct)
Technology	Bioaugmentation Nutrient Addition Electron Donor Addition (Lactate)
Configuration	Groundwater Recirculation
Technology Scale	Field demonstration (pilot proof of technology test)
Media/Matrix Treated	Groundwater
Contaminants Targeted	TCE
Period of Operation	Proof of Technology Test: September 1996 to March 1998 (subject of this case study report) Testing for Technology Scale-up: April 1998 to June 1999 (planned) Full-scale System: Summer 1999 (planned)

Site History/Source of Contamination [1, 2, 5]

Dover Air Force Base (AFB), located in Dover, Delaware, is a 4,000 acre military installation that began operating in 1941. An estimated 23,000 cubic feet of waste, including solvents, waste fuels and oils, and a variety of other wastes, were disposed at the site from 1951 to 1970. Soil and groundwater at the base were found to be contaminated with volatile organic compounds (VOCs), including TCE and PCE, and with heavy metals, including arsenic and cadmium. In March 1989, the site was listed on the National Priorities List. During a remedial investigation, "Area 6" was one of the areas at the base that was determined to have been contaminated with chlorinated solvents; a plume of VOCs was identified in groundwater in this area. Based on the results of that investigation as well as additional sampling, the area was selected for pilot testing of a bioaugmentation process. Concentrations of PCE, TCE, cis-DCE, and vinyl chloride in the pilot area before the test were 46 ug/L, 7,500 ug/L, 1,200 ug/L, and 34 ug/L, respectively.

The remediation of Dover AFB is managed by EPA Region 3 and the Delaware Department of Natural Resources and Environmental Control (DNREC). Records of Decision (RODs) have been signed for nine of the 12 operable units at the site, including operable units within Area 6. Interim RODs were signed in September 1995 that identify the following technologies for remediation at Dover: anaerobic reductive dehalogenation, cometabolic bioventing, and monitored natural attenuation. The pilot test was

performed as part of the Bioremediation Consortium of the Remediation Technology Development Forum.

Geology/Hydrogeology [1, 2, 5]

Dover AFB is underlain by glacial-fluvial deposits of sand, silt, and clay of the Pleistocene Columbia Formation. Within the Area 6 pilot test area, the saturated portion of the formation consists of fine, medium, and coarse sands and is about 38 feet thick. The aquifer acts as one unconfined unit that includes three zones (approximately equal thickness) - an upper zone of fine sand (0 to 12 ft below ground surface or bgs), an intermediate zone of medium sand (12 to 25 ft bgs), and a deep zone also of medium sand (25 to 48 ft bgs). Groundwater is found in the intermediate and deep zones, starting at 10 to 12 ft bgs.

Matrix Characteristics for Area 6 of the Dover AFB Site [1, 5]

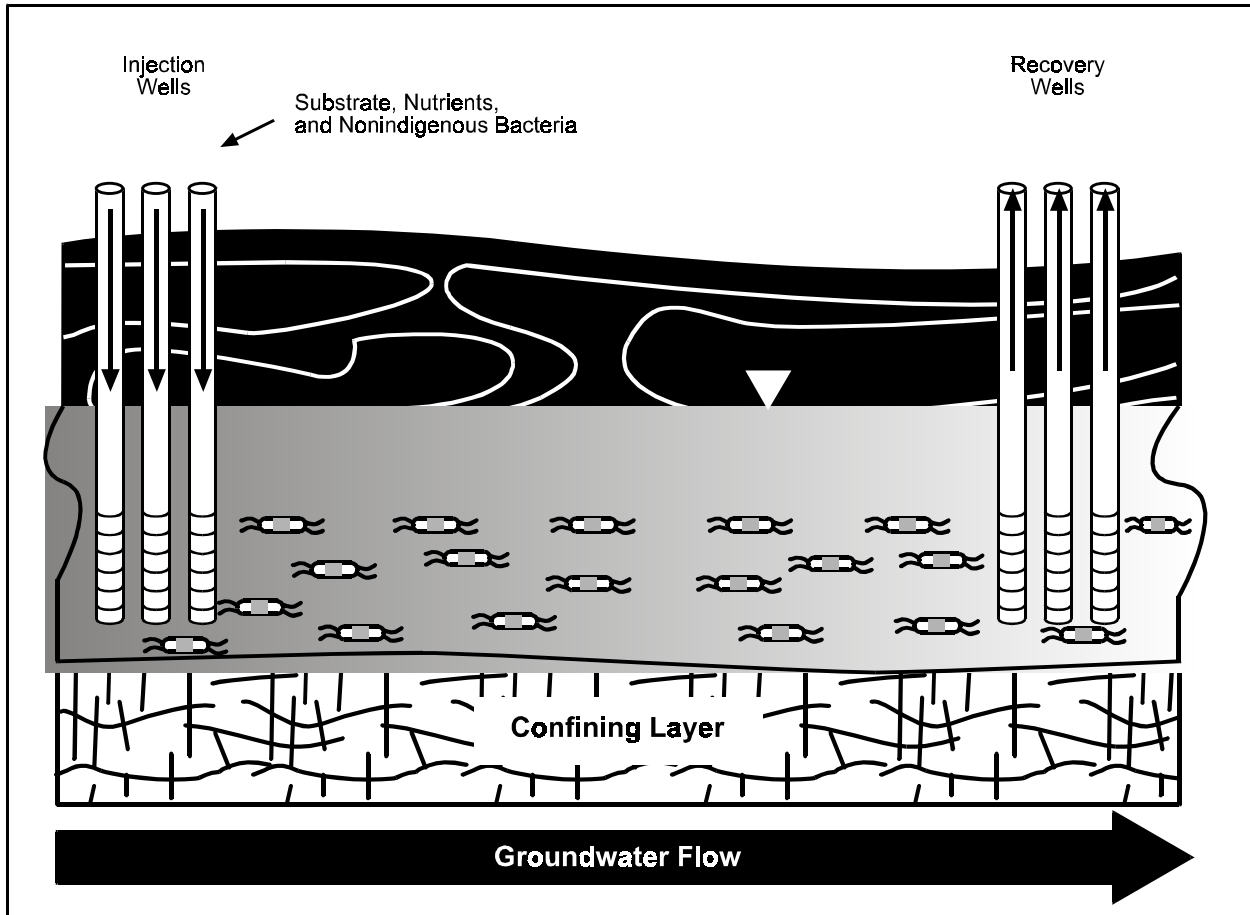
Matrix Characteristic	Value
Soil Type	Sand with varying amounts of clay, silt and gravel. Fine-grained clay and silt to a depth of 5 ft bgs; underlain by more permeable layer of silt and sand.
Soil Permeability	Not provided
Depth to Groundwater	10 to 12 ft bgs
Fraction of Organic Carbon	0.2
Thickness of Aquifer	38 ft (saturated portion)
DNAPL Present	None identified
Hydraulic Conductivity	60 ft/day
pH	5.6 (average)
Porosity	30%
Hydraulic Gradient	0.001 ft/ft
Groundwater Velocity	60 ft/year (0.16 ft/day)

Technology Description [1, 2, 5]

Groundwater flow and three-dimensional transport models (MODFLOW and MT3D) were used in designing the pilot system. The models were used to simulate groundwater flow under different test scenarios and to simulate the three-dimensional transport of substrates.

The pilot system, shown in Figure 1, included three extraction or pumping wells and three injection wells, each screened to a depth of 38 to 48 ft bgs. The pilot system was designed to operate as semi-isolated or “closed-loop” recirculation cell and the wells formed a rectangular, hydraulically-controlled cell that was 40 ft wide and 60 ft long.

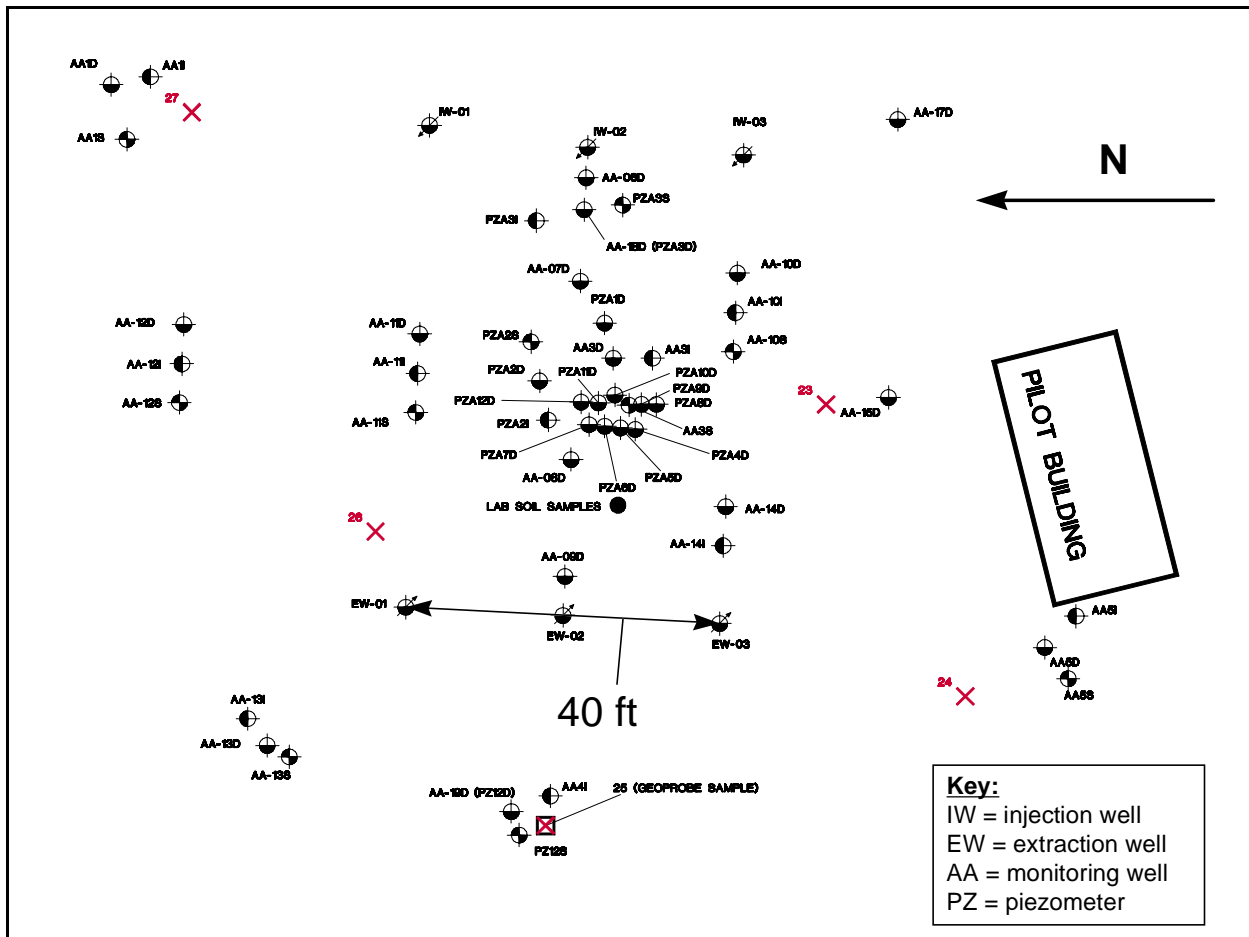
Figure 1: Process Diagram of Accelerated Anaerobic Biodegradation at Dover AFB [3, 5]



The pumping wells were operated at a combined rate of 3.06 gpm (1.03 gpm each), providing a residence time of about 60 days for groundwater from the deep zone of the aquifer.

Monitoring wells, shown in Figure 2, were located within the cell (wells 3D, 6D, 7D, 8D, 9D, and 18D) and outside of the cell. Groundwater samples were collected from the monitoring wells and analyzed for field parameters such as dissolved oxygen and temperature, water chemistry such as total organic carbon and ammonia, injected amendments, and VOCs and degradation products (PCE, TCE, DCE, vinyl chloride, ethene, ethane, and methane). The interior wells were sampled weekly; the exterior wells were sampled monthly. Sampling frequency was determined based on the results of the groundwater modeling. In addition, flow rate, total flow, and pressure were monitored. Prior to the start of the pilot system in September of 1996, a tracer test was conducted to verify groundwater flow patterns, collect data to calibrate and verify modeling results, and to verify system operation.

Figure 2: Well Locations [5]



The system was designed to allow operation with any combination of extraction pumps. The extracted groundwater was combined into a single stream and passed through a filter to remove suspended solids that could cause fouling problems in the injection wells. Using high precision, low volume metering pumps, substrate and nutrients were injected into the combined groundwater stream downstream of the filter. The substrate used in the pilot test was sodium lactate, and for most of the pilot test was delivered as 200 mg/L as carbon to all of the injection wells. The nutrients added to the system were ammonia and phosphate.

Terra Systems, Inc., of Wilmington, Delaware, operated the pilot system at Dover. For the pilot test, a method of cycling substrate and nutrient injection was used to minimize biogrowth in the system tanks and at the well screens. The substrate was fed into the system for 3.75 days, followed by 8 hours of circulation with groundwater (unamended). The nutrient was then injected into the system for 2.75 days, followed by 8 hours of circulation with unamended groundwater. This cycle was used throughout the pilot test.

Technology Performance [1, 2, 5]

The goals of the pilot test, as specified in the design report, were to 1) demonstrate that TCE degradation can be stimulated in the deep portion of an aquifer; 2) confirm that degradation will proceed to nontoxic end products; 3) develop operation and cost data for a full-scale system; and 4) document the methodology used in the pilot system.

The proof of technology portion of the pilot system was operated 18 months, from September 1996 to March 1998. During the first five months of operation, the concentration of TCE in the groundwater gradually decreased, while concentrations of cis-DCE increased slightly. There was no change in the concentrations of vinyl chloride or ethene, indicating that limited dechlorination was occurring.

In January 1997, the substrate feed concentration was increased from 100 to 200 mg/L as carbon in an attempt to stimulate dechlorination. In the monitoring well nearest the injection wells (well 6D), an increase in DCE concentrations was observed almost immediately, along with a decrease in TCE concentrations. Total organic carbon and lactate concentrations also reportedly increased in monitoring wells near and downgradient of the injection wells. However, there was no evidence that dechlorination beyond DCE to vinyl chloride or ethene was occurring. Analyses of the geochemistry of the aquifer before and after test cell operation showed that the indigenous bacteria consumed the lactate; the test cell area showed reductions in nitrate and sulfate concentrations to nondetectable levels and the generation of methane.

In February 1997, a decision was made by the RTDF to evaluate the potential for bioaugmentation for this pilot system. The bioaugmentation plan was approved by EPA Region 3 and DNREC. A number of microcosm tests and column studies were performed with known dechlorinating strains from other sites to evaluate candidate cultures. Based on the screening tests, a culture from the Department of Energy's Pinellas site in Largo, Florida was selected for use at Dover. The culture was grown in liquid media to allow aqueous addition for the pilot test.

Between February and May 1997, TCE continued to degrade to DCE, but the DCE was not further degraded to vinyl chloride. Consequently, the RTDF decided to implement bioaugmentation, in an attempt to further degrade the DCE to vinyl chloride. On June 5, 1997, 180 liters of the aqueous culture (augmenting solution) was injected into the cell through injection well no. 2. On June 20, 1997, another 171 liters of augmenting solution were injected through the same well. Substrate feed concentrations were maintained at 200 mg/L as carbon after bioaugmentation.

For the first 90 days following bioaugmentation, TCE concentrations continued to decrease and DCE concentrations continued to increase; however, there was no evidence of vinyl chloride or ethene in the groundwater. At the beginning of September 1997, vinyl chloride and ethene began appearing in wells closest to the injection wells (well 6D, 18D, and 7D), indicating that DCE degradation was occurring. Vinyl chloride and ethene continued to be detected in downgradient wells within the recirculation cell (wells 3D, 8D, and 9D), and by December 1997, these constituents had been detected in wells inside the recirculation cell but outside the bioaugmented area of the recirculation cell (well 14D and 11D).

Between December 1997 and February 1998, the concentrations of ethene increased, while TCE and DCE concentrations continued to decrease. By March 1998, all TCE and DCE in the groundwater were converted to ethene and between 75 and 80% of the TCE and DCE had been recovered as ethene, indicating that the bioaugmentation was successful in destroying TCE by reductive dechlorination. Reasons for incomplete recovery included biodegradation of ethene or a decline in overall plume contaminant concentrations during the pilot test. Data from a background well upgradient from the pilot

test showed an overall decline in VOC concentrations; however, there was no significant change in the ratio of TCE to DCE in this location.

The proof of technology portion of the pilot test was completed in March 1998. From April 1998 through June 1999, the test is focusing on testing of parameters involved with technology scale up.

Well Plugging During Pilot Test [1]

During the operation of the pilot test, plugging of the injection wells was a problem. As early as 50 days from the start of the system (mid-October 1996), the injection wells became plugged. Biogrowth in the injection wells and on the well screens was the primary cause of the plugging problem. The injection wells were redeveloped and operations resumed. In March 1997, the injection wells again became clogged and another well redevelopment was performed. However, the redevelopment was not as successful as the one performed in October. In an attempt to alleviate the plugging, the substrate was changed to lactic acid in April 1997. However, no appreciable increase in the efficiency of the injection wells was noted and the substrate feed was changed back to sodium lactate on June 8, 1997. Routine brushing of screens and hydrogen peroxide treatments were implemented to keep the wells open.

Future Plans [4, 5]

Funding has been approved for a full-scale application of the technology at an area upgradient of the pilot test. Treatment is scheduled to begin in the summer of 1999. The full-scale system is planned to be a one pass flow through system, with wells used to control the flow of groundwater, directing it through one to two "gates".

The U.S. Army Corps of Engineers (USACE) and Dames and Moore will design the full-scale system.

Technology Cost [2]

Costs for *in situ* enhanced anaerobic biodegradation are presented in Table 1. The total capital costs were \$285,563. The total operating costs were \$164,962 for the first three months of operation (through November 30, 1996) and \$522,620 for the first fifteen months of operation (through November 30, 1997).

According to the RTDF contact, a typical full-scale bioaugmentation system would cost substantially less than the system used in the pilot test at Dover. The pilot system included oversized stainless steel wells, wire-wrapped screens, duplicate control equipment, a monitoring well network that allowed for three-dimensional modeling, a frequent sampling and analysis program, one full-time equivalent operator, and a control building that was designed to be reused to store gymnasium equipment after the project was completed.

Table 1. Costs for *In Situ* Enhanced Anaerobic Biodegradation at Dover AFB [2]

Element	Cost (\$)	
Capital		
Site preparation	5,742	
Structures	39,818	
Process equipment and appurtenances	24,067	
Utility infrastructure hookups (electricity, gas, sewer)	50,716	
Installation labor	8,600	
Monitoring wells	78,306	
Dedicated pumps	18,224	
Other equipment	5,690	
Injection wells	30,400	
Extraction wells	24,000	
Total Capital Costs	285,563	
Operating Costs		
Period	Startup through 11/30/96	Startup through 11/30/97
Direct Labor	74,096	168,850
Materials:		
Substrate	771	6,700
Nutrients	230	580
Supplies	2,272	10,140
Laboratory analysis	75,196	286,000
Sampling	2,018	11,000
Shipping	5,061	22,900
Overhead	975	7,000
Injection well maintenance	4,343	9,450
Total Operating Costs	164,962	522,620

Summary Observations and Lessons Learned [1]

The Dover AFB pilot project was the first successful bioaugmentation project using live bacteria from another site to destroy TCE using reductive dechlorination.

Data from the pilot test indicated that an extended period of time was required for the bacteria to exhibit functional dechlorination. At the start of bioaugmentation, lag periods of about 180 days between bioaugmentation and complete reduction of TCE and DCE to ethene were observed, including a 90-day lag period before vinyl chloride was first observed. The factors that likely contributed to the lag times included slow growth under the conditions at Dover AFB and time required for acclimatization. In addition, the initial mass of bacteria injected during the augmentation (about 35 grams) was relatively small, and may have increased the time required to grow a bacteria population at Dover that was sufficient to dechlorinate the target contaminants.

Laboratory studies are recommended to ensure that the bacterial culture selected will be effective given the site conditions and contaminants. For the Dover pilot test, a number of laboratory studies were performed using soil and groundwater from the Dover site to evaluate candidate cultures. Screening parameters included growth in liquid culture, growth on lactate, and dechlorination to ethene.

Injection well plugging was a problem during the pilot test. Several methods were used to keep the wells unplugged including cleaning the well screens with wire brushes and pumping out residue from the screened interval, using hydrogen peroxide to clean the wells, and changing substrates from sodium lactate to lactic acid. Hydrogen peroxide proved the most effective technique for keeping the wells from clogging.

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Case Study # 9
Cometabolic Bioventing Field Demonstration at Building 719,
Dover Air Force Base, Dover Delaware

Summary Information [1,3,7]

Site Name, Location	Dover Air Force Base, Building 719, Dover, Delaware
EPA ID Number	DE8570024010
Mechanism(s)	Aerobic Oxidation (Cometabolic and Direct)
Technology	Electron Acceptor Addition (Oxygen) Electron Donor Addition (Propane)
Configuration	Direct Injection
Technology Scale	Field Demonstration (Pilot Test)
Media/Matrix Treated	Soil
Contaminants Targeted	TCE, 1,1,1-TCA, <i>cis</i> -1,2-DCE
Period of Operation	Propane acclimation period: December 1997 to April 1998 Bioventing operation: May 1998 to July 1999

Site History/Source of Contamination [1, 2, 3]

Dover Air Force Base (AFB), located in Dover, Delaware, is a 4,000 acre military installation that began operating in 1941. Building 719 is a jet engine inspection and maintenance shop where a variety of materials, including solvents, JP-4 fuel, and hydraulic fluids, have been used in shop operations. Until the mid-1960s, wastes from the shop were discharged to a drainage ditch and sanitary sewer. In addition, the northeast area of the building is the location of two former leaking underground storage tanks (USTs), an oil/water separator, and a former neutralization tank. The results of investigations showed that soil and groundwater in the area of the former USTs were contaminated with fuel (BTEX - benzene, toluene, ethylbenzene, and xylene) and solvents. Results of samples of the vadose zone of Building 719 found concentrations of TCE as high as 250 mg/kg; TCA ranging from 10 to 1,000 mg/kg; and DCE ranging from 1 to 20 mg/kg. TCE concentrations in groundwater were reported as high as 19,000 ug/L.

Dover AFB was listed on the National Priorities List in March 1989. The remediation of Dover AFB is managed by EPA Region 3 and the Delaware Department of Natural Resources and Environmental Control. Interim RODs were signed in September 1995 that identify the following technologies for remediation at Dover: anaerobic reductive dehalogenation, cometabolic bioventing, and monitored natural attenuation. The area in the vicinity of Building 719 was selected for a pilot test of cometabolic bioventing. The cometabolic bioventing pilot test was conducted for the *In Situ* Bioremediation of Chlorinated Solvents Work Group of the Remediation Technology Development Forum (RTDF). The RTDF also conducted a pilot test of bioaugmentation of groundwater at Dover AFB, which is the subject of Case Study number 8.

Geology/Hydrogeology [1,2,7]

Dover AFB is underlain by glacial-fluvial deposits of sand, silt, and clay of the Columbian Formation. The soil in the vicinity of Building 719 was sand with clay, silt, and gravel. Depth to groundwater was 6 to 10 feet (ft) below ground surface (bgs).

Matrix Characteristics for the Building 719, Dover AFB Site [1,6]

Matrix Characteristic	Value
Soil Type	Sand with varying amounts of clay, silt and gravel. Fine-grained clay and silt to a depth of 5 ft bgs; underlain by more permeable layer of silt and sand.
Soil Permeability	1.9×10^{-7} to 7.0×10^{-8} cm ²
Depth to Groundwater	6 to 10 ft bgs
DNAPL Present	None identified
Hydraulic Conductivity	0.017 to 0.052 cm/sec
pH - Soil	6.0 to 11.0 (median 7.7)
Total Chloride	8 mg/kg (median)
Total Kjeldahl Nitrogen	42 mg/kg (median)
Total Phosphorus	30 mg/kg (median)
Total Organic Carbon	0.11% (w/w) (median)

Technology Description and Performance [1,2,4,5,7]

The primary objectives of the pilot test were to determine the efficiency and demonstrate the viability of an *in situ* cometabolic bioventing process for CAHs under field conditions (benzene, toluene, ethylbenzene, and xylene were not targeted for treatment). Prior to the pilot test, laboratory tests were performed on soils from the area of Building 719 to evaluate candidate substrates. Propane was selected because of its ability to stimulate cometabolic activity towards both TCA and TCE.

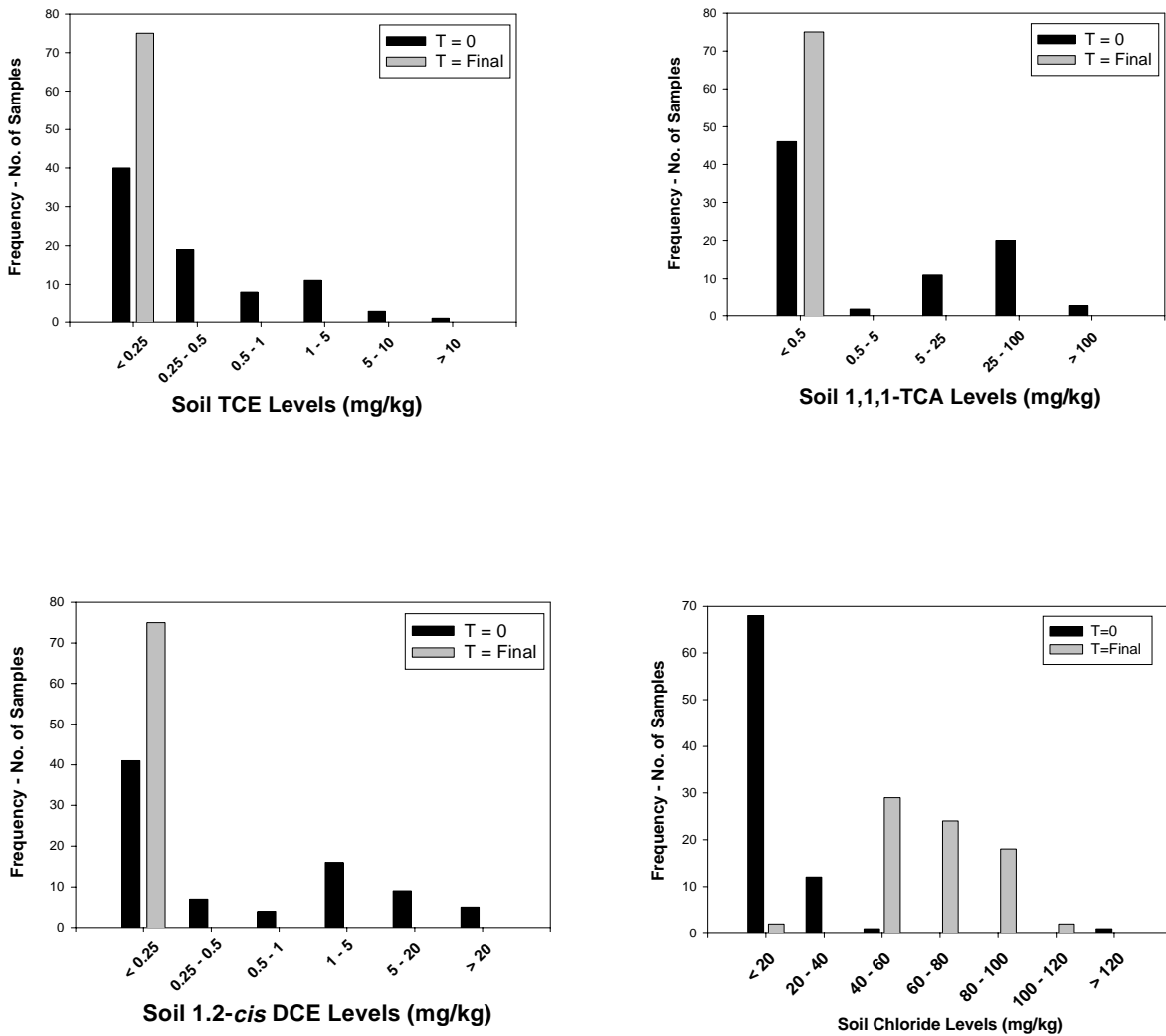
Based on the results of site investigations in the vicinity of Building 719, the location of the test plot was identified as an area of high concentrations of chlorinated aliphatic hydrocarbons (CAHs). The test plot was approximately 30 ft long, 20 ft wide, and 10 ft deep with a volume of 4,500 ft³ of soil. The mass of soil in the test plot was estimated to be 450,000 lbs, based on an assumed density of 100 lbs/ft³. Prior to the pilot test, a total of 80 soil samples were taken from the test plot to provide a contaminant profile and to estimate the mass of contaminants in the test plot. This information was used to develop an order of magnitude estimate of the mass of CAHs and BTEX in the soil for a total gross estimate of 26 lbs of CAH and BTEX constituents in the subsurface soils of the test plot. 1,1,1-TCA made up approximately 70% of the total estimated mass of contaminants.

The pilot system includes three injection wells, screened to a depth of 10 ft bgs, which was the lowest expected water table elevation. In addition, 13 soil gas monitoring points were installed to monitor soil

gas conditions throughout the demonstration. Each of the soil gas monitoring points was equipped with two gas probes (one at a depth of 4-5 ft and one at a depth of 8-9 ft bgs). Another 11 “temporary” soil gas monitoring points were installed for use during initial air permeability testing, and were used during system operation to monitor soil gas. A blower and a mass flow controller were used to inject a mixture of air and propane (300 ppm in air) through the three wells at a rate of 1 cfm.

Figure 1 shows histograms of initial and final concentrations of TCE, TCA, DCE and chloride from the soil in the test plot, including reductions in the concentrations observed for TCE, TCA and DCE during treatment. These reductions can be at least partly attributable to biodegradation by noting the large increase in the soil chloride levels during treatment. Chloride is a product of the biodegradation of chlorinated solvents.

Figure 1. Histograms of initial and final TCE, TCA, DCE and chloride concentrations from soil in the cometabolic bioventing test plot [7]



Technology Cost

Cost data were not available at the time of this report.

Summary Observations and Lessons Learned [7]

The researchers provided the following observations:

- Over a 14-month period of operation, cometabolic bioventing was successful in removing TCE, TCA and DCE from soils in the test plot.
- Laboratory treatability testing identified propane as a useful cosubstrate to drive cometabolism of TCE and TCA (DCE may have been biodegraded via cometabolism or by direct aerobic bioprocesses). The lab studies also successfully predicted the need for a significant time period (weeks) for the test plot to begin using propane after initial exposure. Thus, a cosubstrate acclimation period may be a common element of cometabolic bioventing startup.
- In aerobic bioventing, the amount of fuel biodegraded during treatment can be estimated by oxygen use. In cometabolism, oxygen use and chlorinated solvent biodegradation are not stoichiometrically related. Thus, in cometabolic bioventing, indirect measures must be employed to show that biodegradation is removing contaminant. These may include chloride accumulation in soil, and previous lab studies using site-contaminated soil which have shown that biodegradation of cosubstrate (which can be measured in the field using a shut down test) implies biodegradation of the chlorinated solvent. There is a need for innovative approaches to proving that biodegradation is occurring in the field.

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