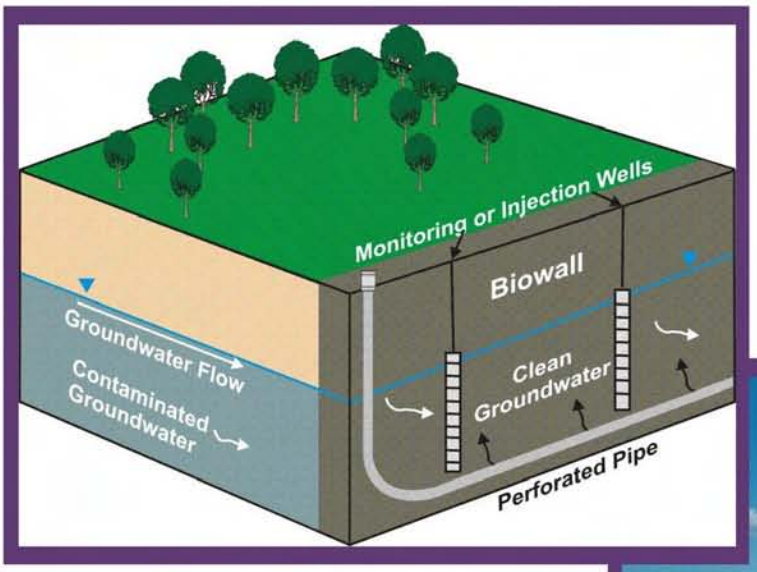
FINAL

Technical Protocol for Enhanced Anaerobic Bioremediation Using Permeable Mulch Biowalls and Bioreactors



U.S. AIR FORCE

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FINAL

TECHNICAL PROTOCOL FOR ENHANCED ANAEROBIC BIOREMEDIATION USING PERMEABLE MULCH BIOWALLS AND BIOREACTORS

May 2008

Prepared for:

Air Force Center for Engineering and the Environment
Technical Directorate
Environmental Science Division
Technology Transfer Outreach Office

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ACRONYMS AND ABBREVIATIONS

µg/day	micrograms per day
µg/kg	micrograms per kilogram
µg/L	micrograms per liter
^{o}C	degrees Celsius
ADF	acid detergent fiber
ADL	acid digestible lignin or acid detergent lignin
AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
Ag/AgCl	silver/silver chloride
ASA	American Society of Agronomy
ASTM	American Society for Testing and Materials
AVS	acid volatile sulfide
atm	atmospheres
bgs	below ground surface
BOD	biological oxygen demand
CA	chloroethane
CAHs	chlorinated aliphatic hydrocarbons
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CES	chromium extractable sulfide
CF	chloroform
CFR	Code of Federal Regulations
CM	chloromethane
cm/day	centimeters per day
cm/sec	centimeters per second
CO_2	carbon dioxide
COD	chemical oxygen demand
CSIA	compound specific isotope analysis
CSM	conceptual site model
CT	carbon tetrachloride
$d^{-1}M^{-1}$	per day per mole
DCA	dichloroethane
DCE	dichloroethene
DCM	dichloromethane
DGGE	denaturing gradient gel electrophoresis
DNAPL	dense non-aqueous phase liquid
DNT	dinitrotoluene
DNX	hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine
DO	dissolved oxygen
DOE	Department of Energy
DOC	dissolved organic carbon
DoD	Department of Defense
DSCR	Defense Supply Center Richmond

Eh	hydrogen electrode
ESTCP	Environmental Security Technology Certification Program
F^0	zero-valent iron, or ZVI
Fe^{2+}	ferrous iron
Fe^{3+}	ferric iron
$FeCO_3$	siderite
Fe_3O_4	magnetite
FeS	iron monosulfide
FeS_2	iron disulfide, for example pyrite
f_{oc}	fraction of organic carbon
FSP	Field Sampling Plan
ft^3/day	cubic feet per day
ft/day	foot per day or feet per day
ft/ft	foot per foot
ft/yr	feet per year
gm/L	grams per liter
gm/cm^3	grams per cubic centimeter
gpm	gallons per minute
GSI	Groundwater Services, Inc.
H_2	molecular hydrogen
H_2S or HS^-	hydrogen sulfide
HASP	Health and Safety Plan
HDPE	high density polyethylene
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine or High-Melting Point Explosive
HRC®	Hydrogen Release Compound®
ID	inside-diameter
IDW	Investigation-derived waste
ITRC	Interstate Technology and Regulatory Council
k	first-order decay or gradation coefficient
K	hydraulic conductivity
kg/day	kilograms per day
kg/L	kilograms per liter
kg/m^3	kilograms per cubic meter
L/day	liters per day
MBT	molecular biological tool
MC	methylene chloride
MCL	maximum contaminant level
mg/L	milligrams per liter
mg/kg	milligrams per kilogram
ml	milliliter
Mn^{4+}	manganese (insoluble)
Mn^{2+}	di-valent manganese (soluble)
MNA	monitored natural attenuation
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
mol/L	moles per liter

MS	matrix spike
MSD	matrix spike duplicate
mV	millivolts
NDF	neutral detergent fiber
n_e	effective porosity
NFTA	National Forage Testing Association
NIRS	near infrared reflectance spectroscopy
nmol	nanomoles
nmol/L	nanomoles per liter
NRMRL/GWERD	National Risk Management Research Laboratory/Ground Water and Ecosystems Restoration Division (USEPA)
NWIRP	Naval Weapons Industrial Reserve Plant
O&M	operation and maintenance
OD	outside-diameter
OPS	Operating Properly and Successfully
ORP	oxidation-reduction potential
OSD	Office of the Secretary of Defense
Parsons	Parsons Infrastructure & Technology, Inc.
PCBs	polychlorinated biphenyls
PCD	Pueblo Chemical Depot
PCE	tetrachloroethene (or perchloroethene)
PID	photoionization detector
PPE	personal protective equipment
psi	pounds per square inch
PRG	Preliminary Remediation Goal
PVC	polyvinyl chloride
q	specific discharge or Darcy's velocity
Q	volumetric flow rate
QA/QC	quality assurance / quality control
QAPP	quality assurance project plan
RCRA	Resource Conservation and Recovery Act
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine or Royal Demolition Explosive
redox	reduction-oxidation
ROD	Record of Decision
RPM	restoration or remedial project manager
S^o	elemental sulfur
SAP	Sampling and Analysis Plan
SAS	strong acid extractable
SHE	standard hydrogen electrode
SO_4^{2-}	sulfate
SOP	standard operating procedure
T-RFLP	terminal restriction fragment length polymorphism
TAT	triaminotoluene
TCA	trichloroethene
TCE	trichloroethene
TCEQ	Texas Commission on Environmental Quality

TDS	total dissolved solids
TEAP	terminal electron accepting process
TNT	2,4,6-trinitrotoluene
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
TOC	total organic carbon
USEPA	United States Environmental Protection Agency
v	average linear velocity or seepage velocity
VC	vinyl chloride
VFAs	volatile fatty acids
VOA	volatile organic analysis
VOC	volatile organic compound
WAS	weak acid extractable
ZVI	zero-valent iron

SECTION 1

INTRODUCTION

1.1 PROBLEM STATEMENT

The Department of Defense (DoD) has identified perhaps thousands of sites where groundwater is contaminated with chlorinated solvents, perchlorate, and explosive compounds. Remediation of these contaminants in groundwater is problematic, and these groundwater contaminant plumes represent one of the DoD's largest remediation liabilities. Enhanced *in situ* anaerobic bioremediation can be an effective method of degrading chlorinated solvents and other contaminants in groundwater subject to anaerobic transformation. Advantages of enhanced *in situ* anaerobic bioremediation include complete mineralization of the contaminants *in situ* with low operation and maintenance (O&M) requirements and lower cost for materials compared to more active, engineered remedial systems.

Permeable mulch biowalls are an increasingly employed approach to applying enhanced *in situ* anaerobic bioremediation. A biowall trench physically cuts though and removes a portion of the aquifer matrix, allowing for uniform distribution of substrate and contact with contaminated groundwater flowing through the biowall treatment zone (**Figure 1.1**). Biowall substrates are typically low cost materials such as mulch and compost, and common construction materials such as sand and gravel are used to prevent compaction and maintain permeability. Biowall materials may be modified to include amendments to stimulate both biotic and abiotic degradation processes, allowing the practitioner to optimize biowall performance based on the type of contaminant(s) present and the desired degradation pathway(s) to be stimulated. The technology may also be applied in source areas or to capture "deeper" (*e.g.*, greater than 35 feet below ground surface [bgs]) plumes in an *in situ* bioreactor configuration using recirculation of groundwater.

Permeable mulch biowalls and bioreactors are being used by the DoD and industry to remediate shallow groundwater contaminated with dissolved chlorinated solvents, perchlorate, and explosives. The use of low cost substrate materials and the low O&M requirements of a biowall or bioreactor treatment system may result in cost savings over more highly engineered remedial systems. Application of the technology is not limited to passive biowalls to treat dissolved contaminant plumes. The use of recirculation to pass contaminated groundwater through a permeable mulch bioreactor or infiltration trench provides an alternative method to treat source areas.

Permeable mulch biowalls and *in situ* bioreactors hold great promise as a remedy for shallow groundwater contaminant plumes and some source areas. This technical protocol has been prepared by the Air Force Center for Engineering and the Environment (AFCEE) to provide guidance on the design and implementation of permeable mulch biowalls and bioreactors for enhanced *in situ* bioremediation of contaminants subject to anaerobic transformation in groundwater.

Figure 1.1 Installation of a Permeable Mulch Biowall at Altus AFB, Oklahoma

1.2 OBJECTIVES

This protocol describes the scientific and technical basis for use of enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls and *in situ* bioreactors to promote the appropriate use of the technology. This document is intended to provide restoration project managers (RPMs) and their contractors with the information necessary to make informed decisions about implementing the technology, and to select specific approaches that are suitable for achieving remedial goals and performance objectives. This document builds on the scientific basis for enhanced anaerobic bioremediation of chlorinated solvents, and the methods for determining whether a site is suitable for the technology, that are described in the *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* (AFCEE *et al*., 2004).

Guidance is provided on technology selection, site screening, design criteria, installation methods, performance monitoring, and data interpretation for the various engineered approaches currently being used. Not all sites are suitable for application of the technology, and site conditions dictate which biowall or bioreactor designs will be effective. Therefore, this protocol is intended to assist the practitioner in recognizing potential biowall/bioreactor sites where the probability of success is high, and to assist in appropriate design and application of the remedy.

1.3 ORGANIZATION OF THE BIOWALL PROTOCOL

This protocol is divided into eleven sections and six appendices. **Section 1** provides an overview of the use of permeable mulch biowalls and *in situ* bioreactors to degrade various contaminants subject to anaerobic degradation processes. **Section 2** describes remedial objectives that the technology may address, and provides a preliminary screening evaluation useful for determining if the technology is appropriate for a site.

Section 3 provides design and engineering considerations for construction of biowall and bioreactor systems. **Section 4** provides guidance on developing and implementing a construction management plan. **Section 5** describes development of a residuals management plan. **Section 6** provides an example field sampling plan (FSP) that can be used to ensure quality and to evaluate application of the technology, while **Section 7** provides considerations for data interpretation and performance evaluation. **Section 8** provides guidance on developing long-term O&M plans. **Section 9** contains an evaluation of the cost to install and monitor

biowall and bioreactor systems. **Section 10** contains a summary with a discussion of future directions for applying biowalls and bioreactors, and **Section 11** contains references cited throughout this document.

Appendix A contains contact information for key project personnel involved in the generation of this document. **Appendix B** describes degradation processes for contaminants targeted for anaerobic bioremediation using permeable mulch biowalls and bioreactors. **Appendix C** provides reference tables for properties of potential contaminants and their degradation products. **Appendix D** provides example methods and calculations for evaluating the potential for reactive iron sulfides to form, and to evaluate whether inorganic amendments should be added to stimulate biogeochemical transformation of chlorinated solvents. **Appendix E** provides reference calculations useful for the design of horizontal delivery pipes for fluid substrate amendments. **Appendix F** provides three example case studies evaluating the performance of biowall and bioreactor systems.

1.4 TECHNOLOGY DESCRIPTION

The AFCEE and DoD are demonstrating the use of permeable mulch biowalls and bioreactors for enhanced *in situ* anaerobic bioremediation of chlorinated solvents, perchlorate, and explosives in groundwater. Biowall trench systems using mulch and compost are intended to provide a long-term source of organic carbon to stimulate anaerobic degradation of contaminants in groundwater. Biowalls have been shown to remain effective for several years without any modification or amendments (*e.g.*, **Appendix F.2**) The ability to replenish a biowall or bioreactor with fluid substrates may allow the treatment system to be effective for periods of 5 to 10 years or more.

Solid phase substrates used in biowalls and bioreactors include mulch and compost. Mulch is generally obtained from shredding and chipping of tree and shrub trimmings and is primarily composed of cellulose and lignin. Often "soft" plant material or compost is incorporated to provide a source of more readily degradable organic carbon and a source of nutrients for microbial growth. Degradation of the substrate by microbial processes in the subsurface provides a number of breakdown products, including metabolic and humic acids, which act as secondary organic substrates and electron shuttles (Kwon and Finneran, 2006; Ahmad *et al.*, 2007b).

Sand and gravel are also used in biowalls and bioreactors to maintain permeability and prevent compaction. Inorganic amendments such as ferric iron and sulfate may also be added to stimulate the formation of reduced metal sulfides for abiotic degradation of chlorinated solvents.

1.4.1 Remedial Objectives

Biowalls are typically installed in trenches oriented perpendicular to groundwater flow to intercept contaminated groundwater. *In situ* bioreactors may be installed in source area excavations, where contaminated groundwater is recirculated through the treatment media, and leaching of soluble organic carbon extends the treatment zone into the saturated zone below the bioreactor cell. Recirculation has also been used to pass contaminated groundwater through permeable mulch infiltration trenches.

Biowalls are used to intercept and treat groundwater contaminant plumes. An alternative for source area treatment is to use mulch and compost in a recirculating bioreactor

Plume Containment. For large plumes having poorly defined, widely distributed, or inaccessible source areas, an enhanced bioremediation system may be configured as a permeable mulch biowall to intercept and treat a groundwater contaminant plume (**Figure 1.2**). For example, biowalls may be employed upgradient of a property boundary or point of regulatory compliance to prevent plume migration to potential receptors. Contaminant mass is delivered to the treatment zone through advective groundwater flow under a natural hydraulic gradient. Two case studies of biowall applications are included in **Appendix F.1** and **Appendix F.2**. A summary table of DoD and industry biowall applications is included in **Section 1.4.4**.

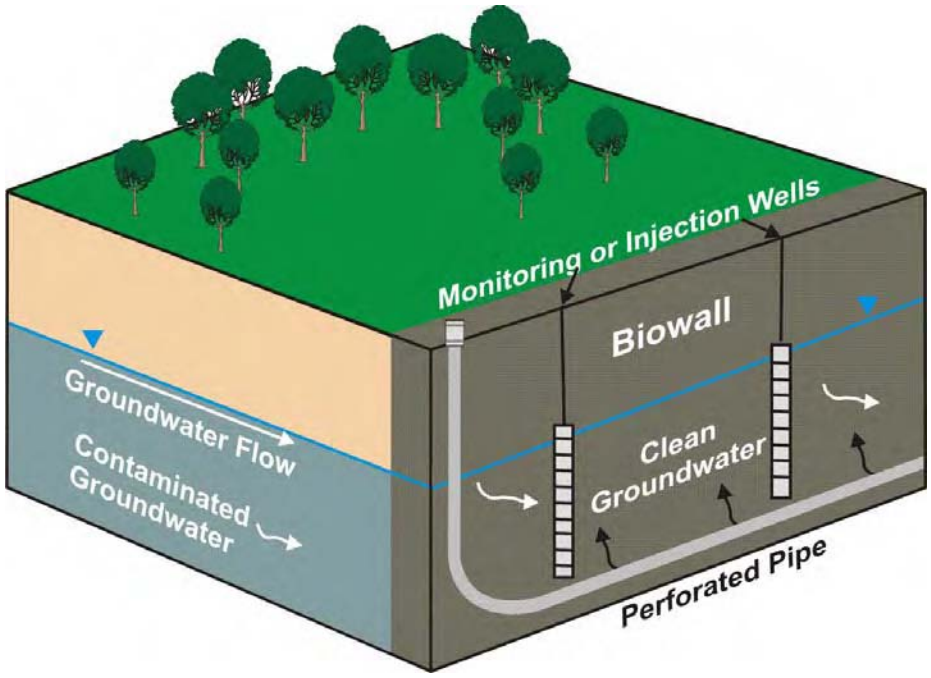

Figure 1.2 Schematic of a Permeable Mulch Biowall

O&M of a biowall is limited to infrequent injections of fluid substrates (*e.g.*, emulsified vegetable oil) to replenish the supply of organic carbon in the biowall. The frequency of biowall replenishment is site-specific, typically on the order of every 3 to 5 years. If the source of the contaminants upgradient of the biowall is not addressed (*e.g.*, the contaminant load to the biowall system), the period of operation could be indefinite and life-cycle cost could be significant.

Source Zone Treatment. *In situ* permeable mulch bioreactors have been installed to address source zones in landfill settings (**Appendix F.3**, Parsons, 2006a). The objectives of source area treatment are to accelerate destruction of contaminant mass within the source zone and to limit mass discharge from the source by reducing concentrations of contaminants that migrate in a downgradient direction. A summary table of DoD bioreactor applications is included in **Section 1.4.4**.

Bioreactors constructed of mulch and compost are based on the same principles of enhancing anaerobic biodegradation processes as biowalls. They offer a low-cost alternative for source area treatment, particularly when installed as part of planned source area excavation activities. Excavation is often used to remove contaminated soils from the subsurface. Installing a recirculating bioreactor within the excavation is useful to treat contaminated soil and groundwater beneath the water table (**Figure 1.3**). Recirculation allows for capture of contaminated groundwater downgradient of the bioreactor, with the groundwater recirculated through the bioreactor materials. The recirculated groundwater also carries soluble organic carbon back into the aquifer, which allows for treatment of a much greater volume of the aquifer relative to the volume of the constructed bioreactor cell (**Section 3.2.2**). **Appendix F.3** provides a case study of a bioreactor demonstration at Landfill 3, Altus Air Force Base (AFB), Oklahoma.

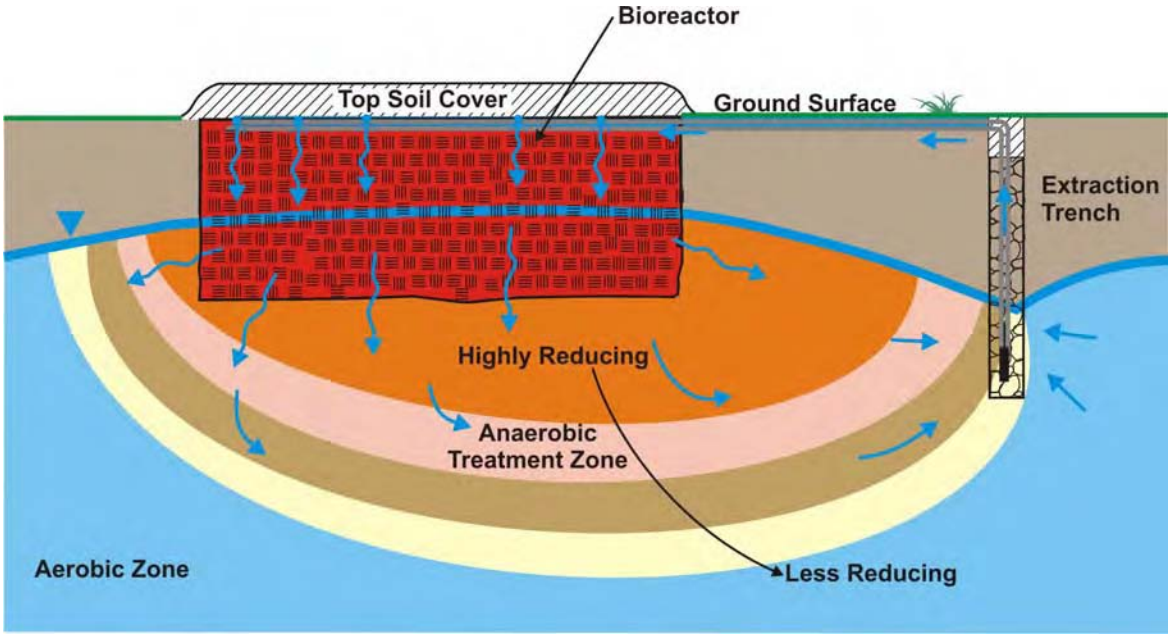

Figure 1.3 Schematic of a Permeable Mulch Bioreactor (modified from Parsons, 2006a)

1.4.2 Applicable Contaminants

Contaminants in groundwater that are amenable to anaerobic degradation processes that may be treated with permeable mulch biowalls and *in situ* bioreactors include the following:

- Chlorinated aliphatic hydrocarbons (CAHs, or chlorinated solvents) such as tetrachloroethene (PCE), trichloroethene (TCE), 1,1,1-trichloroethane (1,1,1-TCA), or carbon tetrachloride (CT) (see **Table C.1A** in **Appendix C** for a detailed list of compounds);
- Oxidizers such as perchlorate and chlorate (**Table C.1B**);
- Explosive and ordnance compounds such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and 2,4.6-trinitrotoluene (TNT) (**Table C.1C**);
- Dissolved metals such as hexavalent chromium;

- Nitrate and sulfate; and
- *Potentially* chlorinated pesticides (*e.g.*, chlordane), polychlorinated biphenyls (PCBs), pentachlorophenol, and fluorohydrocarbons (Freon).

Many of the techniques described in this protocol to create anaerobic reactive zones for chlorinated solvents may also be applicable to the design and implementation of enhanced anaerobic bioremediation systems for the constituents listed above.

1.4.3 Anaerobic Degradation Processes for Chlorinated Solvents

Air Force applications of permeable mulch biowalls to date have focused on the remediation of CAHs, primarily PCE, TCE, dichloroethene (DCE) isomers, and vinyl chloride (VC). Biowalls stimulate anaerobic degradation processes, which for CAHs may include 1) biotic anaerobic reductive dechlorination, 2) biotic anaerobic oxidation (DCE and VC), and 3) abiotic dechlorination by reaction with reduced metal sulfides or green rusts (*e.g.*, see **Appendix B**). Most biowalls installed to date are primarily intended to stimulate biotic reductive dechlorination; however, these processes are not exclusive of each other and in some cases all three degradation reactions may be occurring (AFCEE *et al.*, 2008).

Three primary anaerobic transformation processes are thought to commonly occur in mulch biowalls and bioreactors:

1. ***Biotic reductive dechlorination,***
2. ***Anaerobic oxidation, and***
3. ***Abiotic dechlorination.***

It may be difficult to differentiate among these processes using conventional monitoring and analytical methods.

In practice, organic substrates are fermented to molecular hydrogen (H_2) and low-molecular-weight fatty acids. These short-chain molecules (such as acetate, propionate, and butyrate) in turn provide carbon and energy to microorganisms which facilitate biotic reductive dechlorination. In the biotic reductive dechlorination process, microorganisms sequentially replace chlorine atoms with hydrogen, forming more reduced dechlorination products. For example, the chlorinated ethenes are transformed sequentially from PCE to TCE to DCE to VC to ethene as shown on Pathway 1 on **Figure 1.4**. Ethene may be further reduced to ethane, or ethene and ethane may degrade to carbon dioxide and water. If the microorganisms are able to obtain metabolically useful energy from reductive dechlorination, this process is referred to as dehalorespiration or halorespiration (United States Environmental Protection Agency [USEPA], 2000b and AFCEE *et al*., 2004).

Abiotic dechlorination by reactive metal-sulfide minerals (*e.g.*, iron-monosulfide) may be a primary degradation pathway for CAHs at sites such as the biowall systems at Altus AFB, Oklahoma (**Appendix F.2**) and Dover AFB, Delaware (Parsons, 2007a). When the process is a result of both biological and geochemical processes it is referred to as ***in situ biogeochemical transformation***. Pathway 2 in **Figure 1.4** illustrates abiotic reduction of chlorinated ethenes by reaction with iron monosulfide (FeS). Reactive metal-sulfide minerals may be created as a result of the anaerobic biological processes of iron and sulfate reduction, where the abiotic degradation of CAHs is an indirect result of substrate addition. Other minerals that may react with CAHs include sulfate green rusts (Lee and Batchelor, 2002b) or magnetite (Ferrey *et al.*, 2004). Additional discussion of biogeochemical transformation processes for CAHs can be found in **Appendix B** and in AFCEE *et al.* (2008).

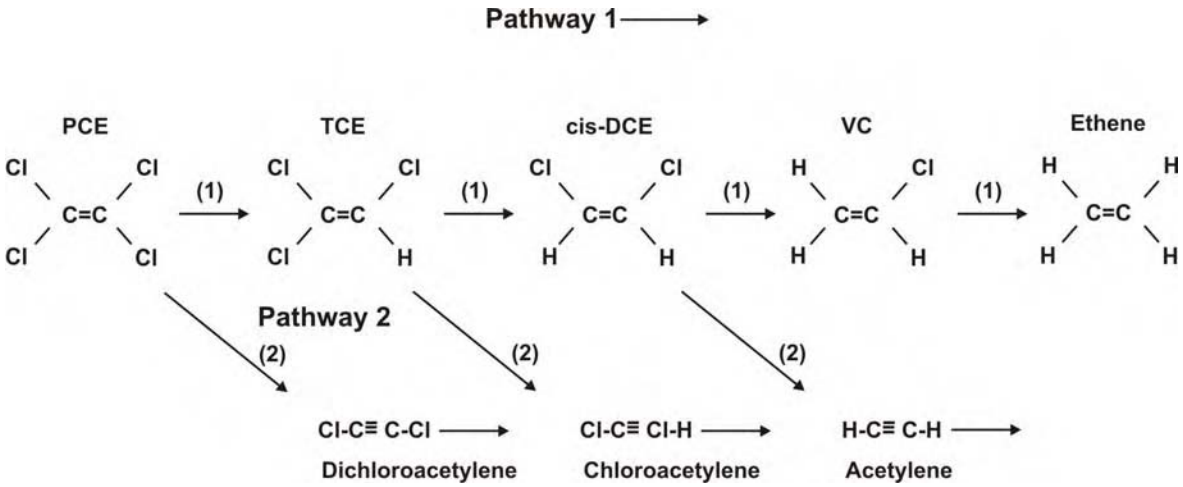

Figure 1.4 Pathways for (1) Biotic Transformation of Chlorinated Ethenes and (2) Abiotic Transformation by Iron Monosulfide (modified from Butler and Hayes, 2001)

In some cases, biowall or bioreactor materials may be modified by addition of sulfate or iron to stimulate the biogeochemical transformation process. For example, magnetite ore has been added to biowall segments at Altus AFB, Oklahoma and Ellsworth AFB, South Dakota on an experimental basis, while powdered gypsum and gypsum fertilizer pellets have been added to biowall segments at Dover AFB, Delaware and Ellsworth AFB, South Dakota.

Humic acids and other compounds leached from mulch/compost material have been shown to function as electron shuttles. For example, Nevin and Lovley (2000) identified several humic acids that act as electron shuttles to facilitate the abiotic reduction of ferric iron to form iron sulfides under anaerobic conditions. In addition, humic acids in mulch and compost mixtures may also serve as electron acceptors in energy yielding reactions that result in the oxidation of DCE and VC under anaerobic conditions (*e.g.*, Bradley *et al.*, 1998a and 1998b). Anaerobic oxidation of DCE and VC produces carbon dioxide and chloride, which may be naturally present at concentrations that prevent differentiation of this degradation process.

It may be difficult to differentiate among different degradation processes using conventional monitoring and analytical methods (AFCEE *et al.* , 2008). The DoD and USEPA (*e.g.*, Shen and Wilson, 2007) are researching ways to differentiate between degradation pathways. **Appendix B** provides a more detailed description of the biodegradation processes for CAHs, as well as a description of degradation processes for perchlorate and explosive compounds.

Biowalls have been installed in at least 13 facilities in 11 States, covering five USEPA Regions. In addition to CAHs, biowalls have been installed to treat perchlorate and explosive compounds.

Two recirculating bioreactors have been installed at Altus AFB, Oklahoma, and one recirculating bioreactor has been installed at Camp Stanley, Texas.

1.4.4 Biowall and Bioreactor Applications

To date, permeable mulch biowalls and *in situ* bioreactors have been installed at over a dozen DoD facilities. **Table 1.1** provides a summary list of biowall and bioreactor applications. Locations of DoD applications are shown on **Figure 1.5**. Results

Table 1.1
Summary of Permeable Mulch Biowall and Bioreactor Applications

Site	Location	Installation Date	Dimensions	Contaminants (maximum concentrations in micrograms per liter)	References/Notes
Air Force					
B301 Biowall	Offutt AFB, Nebraska	January 1999 (pilot)	100 feet long by 23 feet deep by 1.0 feet wide	TCE (1,000 µg/L) DCE (290 µg/L) VC (13 µg/L)	Groundwater Services, Inc. (GSI), 2001
B301 Biowall	Offutt AFB, Nebraska	July 2001 (full-scale)	500 feet long by 25 feet deep by 1.5 feet wide	TCE (1,100 µg/L) DCE (290 µg/L) VC (13 µg/L)	GSI, 2004
Operable Unit 1 Biowall	Altus AFB, Oklahoma	July 2002 (pilot)	455 feet long by 24 feet deep by 1.5 feet wide	TCE (8,000 µg/L) DCE (1,800 µg/L)	Appendix F.2; Parsons, 2007c; Kennedy and Everett, 2003
SS-17, SS-18, SS-23 Biowalls	Altus AFB, Oklahoma	March-May 2005 (full-scale)	Approximately 5,400 linear feet in six sections, ranging from 32 to 35 feet deep by 2 feet wide	TCE (31,800 µg/L) DCE (6,400 µg/L) VC (11,600 µg/L)	Parsons, 2007d. Maximum concentrations at Section F in July and October 2005 (post-installation)
Building 506 Bioreactor	Altus AFB, Oklahoma	May 2007 - Recirculation started July 2007	Approximately 90 feet by 70 feet. Bench at 20 feet deep, with maximum depth to 37 feet deep.	TCE (36,800 µg/L) DCE (2,310 µg/L) VC (10,900 µg/L) Maximum concentrations prior to recirculation.	Parsons, 2008. Recirculating bioreactor using groundwater extracted from Section F1 and Section F2 of the Altus AFB SS-17 biowall system.
DP-32 Biowall	Whiteman AFB, Missouri	March 2004	270 feet long by 10 to 20 feet deep by 3 feet wide	TCE (>1,000 µg/L) DCE (234 µg/L) VC (trace)	CH2M Hill, 2004
Zone D Biowall	FE Warren AFB, Wyoming	August 2004	150 feet long by 25 feet deep by 1.5 feet wide	TCE (220 µg/L) DCE (2.4 µg/L)	Parsons, 2007b
Landfill 3 Biowall	Air Force Plant 4, Texas	October 2004	90 feet long by 7-10 feet deep by 2 feet wide	TCE (253 µg/L) DCE (590 µg/L)	Wice *et al.*, 2006
WP-14 Biowall	Dover AFB, Delaware	December 2004	Dual Biowall: 250 feet long by 25 feet deep by 2 feet wide	PCE (3,400 µg/L) TCE (930 µg/L) DCE (2,000 µg/L) VC (63 µg/L)	Parsons, 2007a

Table 1.1 (continued)
Summary of Permeable Mulch Biowall and Bioreactor Applications

Site	Location	Installation Date	Dimensions	Contaminants (maximum concentrations in micrograms per liter)	References/Notes
BG05 Biowall	Ellsworth AFB, South Dakota	June 2005	580 feet long by 32 feet deep by 2 feet wide	TCE (176 µg/L)	Parsons, 2005 and 2006c
Site 10 Bioreactor	Buckley Air Force Base, Colorado	November 2005	Passive bioreactor – source area excavation lined with a 1-foot thick mulch/soybean oil mixture.	PCE (11,934 µg/L) TCE (770 µg/L) DCE (834 µg/L) VC (106 µg/L)	Parsons, 2006d. Piping installed for future substrate injections.
OU7 – Pit 1 and Pit 2 Biowalls	Defense Supply Center Richmond (DSCR),Virginia	August 2007	Two biowalls per plume ranging from 45 to 115 feet long, by 20 to 25 feet deep, by 2 feet wide	PCE (155,000 µg/L) TCE (12,200 µg/L) DCE (1,440 µg/L) VC (913 µg/L)	Leeper, *et al.*, 2007
Navy					
Area M, Area S, and Area F Biowalls	Naval Weapons Industrial Reserve Plant (NWIRP) McGregor, Texas	1999 to 2005	Over 12,000 linear feet to depths of 15 feet	Perchlorate (27,000 µg/L) TCE (500 µg/L)	Cowan, 2000; Perlmutter *et al*., 2000 and 2001; EnSafe, 2005
Army					
Ash Landfill Biowall	Seneca Army Depot, New York	August 2005 (pilot)	Dual Biowall 200 feet long by 32 feet deep by 2 feet wide	TCE (860 µg/L) DCE (980 µg/L) VC (86 µg/L)	Appendix F.1
Ash Landfill Biowalls	Seneca Army Depot, New York	October 2006 (full-scale)	Single Double-Wide and Dual Biowall System 1,500 linear feet, 10 to 15 feet deep by 3 to 6 feet wide	TCE (2,000 µg/L) DCE (960 µg/L) VC (95 µg/L)	Appendix F.1
Western Industrial Area Biowalls	Red River Army Depot, Texas	April 2007 (full-scale)	North Biowall: 190 feet long by 35 feet deep by 2 feet wide. South Biowall: 210 feet long by 35 feet deep by 2 feet wide	TCE (10,000 to 15,000 µg/L) (North Biowall)	Ahmad, F., 2007 (personal communication).

Table 1.1 (concluded)
Summary of Permeable Mulch Biowall and Bioreactor Applications

Site	Location	Installation Date	Dimensions	Contaminants (maximum concentrations in micrograms per liter)	References/Notes
Army (continued)					
SWMU-B3 Bioreactor	Camp Stanley Storage Activity, Texas	November 2006	Recirculating Bioreactor Seven landfill trenches	DCE (250 µg/L)	Recirculation operational February 2007
ESTCP					
Landfill 3 Bioreactor (with AFCEE)	Altus AFB, Oklahoma	November 2003	Landfill Bioreactor: 30 feet by 30 feet by 11 feet deep	TCE (14,060 µg/L) DCE (1,629 µg/L)	Parsons, 2003, 2006a, and 2007e Appendix F.3
SWMU-17 Biowall	Pueblo Army Depot, Colorado	November 2005	Single Biowall	RDX (>50 µg/L)	ESTCP ER-0426; GSI, 2005
Industrial					
Confidential Industrial Site, Biowalls	Virginia	September 2002	Two Biowall Trenches: Trench 1 - 140 feet long by 15 feet deep by 3 feet wide; Trench 2 - 120 feet long by 13 feet deep by 3 feet wide	PCE (390 µg/L) TCE (42 µg/L) Perchlorate (846 µg/L)	Morris, K., 2007 (personal communication). Downgradient extraction pump recirculated into Trench 1 at average rate of 2 gpm.
Confidential Industrial Site, Biowall Infiltration Trenches	Arkansas	August 2003	Biowall Infiltration Trench: 180 feet long by 3 feet wide by 17 to 20 feet deep.	Perchlorate (452,000 µg/L)	Smith and Morris, 2007 Morris, K., 2007 (personal communication).
Confidential Industrial RCRA Site Biowalls	Arkansas	December 2006	Four 400-foot segments to 25 feet deep	Perchlorate (800 to 1,300 µg/L)	Morris, K., 2007 (personal communication).

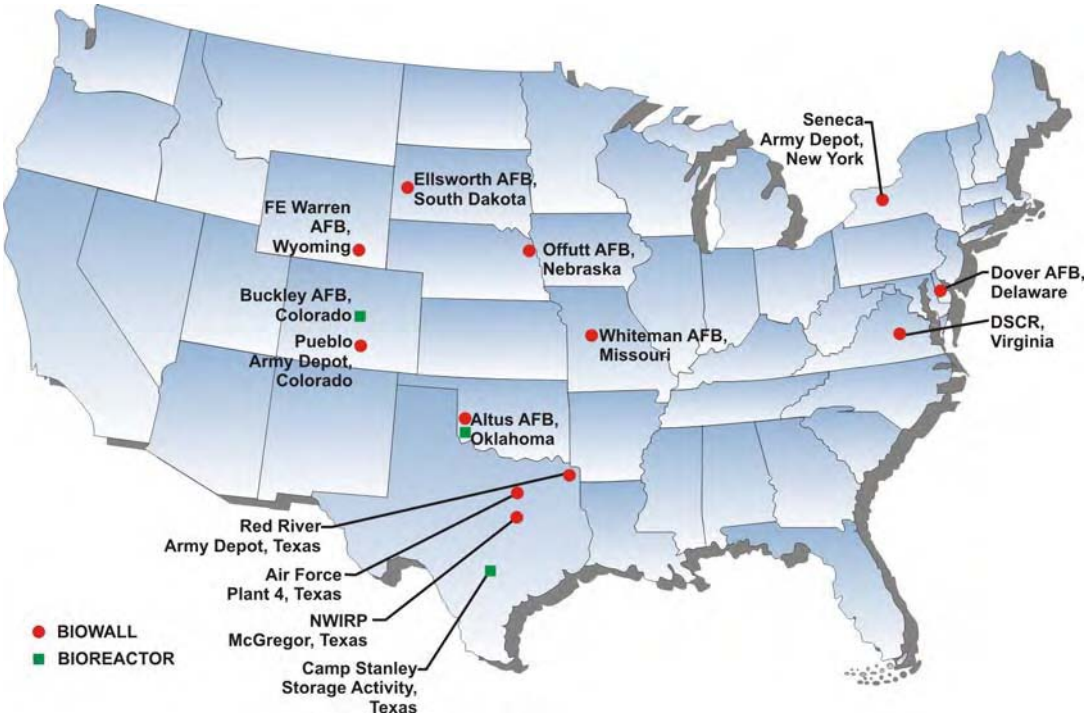

Figure 1.5 Location of DOD Biowall and Bioreactor Applications

of biowall demonstrations at Offutt AFB, Nebraska, and Altus AFB, Oklahoma, have been used to design and implement full-scale systems. At Altus AFB, over 5,000 linear feet (1,500 meters) of biowall was installed as a containment measure along the southern base boundary. Additional Air Force biowall demonstrations for CAHs are ongoing at Dover AFB, Delaware; F.E. Warren AFB, Wyoming; Whiteman AFB, Missouri; Air Force Plant 4, Texas; and Ellsworth AFB, South Dakota.

The Navy has installed over 12,000 linear feet of permeable mulch biowalls since 1999 for the remediation of perchlorate at the former Naval Weapons Industrial Reserve Plant (NWIRP) McGregor, Texas. The biowall system covers several large perchlorate plumes that are up to several thousand feet in length. Some perchlorate plumes are commingled with CAHs, primarily TCE. Based in part on a designation of the biowall system as Operating Properly and Successfully (OPS), the Navy was able to transfer the entire 39 square kilometer (9,700 acre) property to the City of McGregor in November 2006 for redevelopment (CH2M Hill, 2006).

The Office of the Secretary of Defense (OSD) Environmental Security Technology Certification Program (ESTCP), in conjunction with the Army, is demonstrating the use of a permeable mulch biowall for energetics at the Pueblo Army Depot, Colorado. ESTCP and the Air Force have demonstrated a bioreactor for CAHs at Altus AFB, Oklahoma. The Army has also installed a recirculating bioreactor system for a landfill site at Camp Stanley, Texas.

1.4.5 Advantages and Limitations of Permeable Mulch Biowalls and Bioreactors

Advantages of enhanced anaerobic bioremediation include the potential for complete destruction of dissolved contaminant mass *in situ* with lower capital and maintenance costs relative to other highly engineered remedial technologies, with potential application to a wide

variety of contaminants. For example, the use of permeable mulch biowalls and bioreactors provides a low-cost alternative to other reactive barrier systems such as zero-valent iron (ZVI) walls. Based on the cost of zero-valent iron over the past five years, biowall construction costs are typically one-third to one-fourth the cost of equivalent iron walls. Other advantages, as well as limitations, of permeable mulch biowalls and bioreactors are summarized below.

Advantages of Permeable Mulch Biowalls and In Situ Bioreactors

Remediation of contaminants in groundwater is difficult and sometimes technically infeasible due to aquifer heterogeneity and the recalcitrance of the contaminant compounds. Highly engineered remedial techniques such as pump-and-treat are costly due to inherent mass transfer limitations, capital expenditures, the need for treatment of secondary waste streams, energy consumption, and long-term O&M requirements. Conversely, enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls and bioreactors may in some cases offer the following advantages:

- **Barriers to Contaminant Migration.** Biowalls are effective for shallow groundwater plumes to maximum depths of 30 to 35 feet in low to moderate permeability or highly heterogeneous formations. Biowalls installed in higher permeability formations require additional considerations to achieve the desired treatment. The continuity of the trench reduces the potential for groundwater bypass due to preferential flow paths, or non-uniform distribution of substrate that may occur with delivery of fluid substrates using injection wells. In addition to the biowall proper, the effective reaction zone may extend downgradient of the biowall trench due to release and migration of soluble organic carbon.

- **Source Area Treatment.** Bioreactors are an alternative treatment approach for source areas where source removal via excavation is being considered. Combined with recirculation of groundwater, a bioreactor may treat an area much greater than the limited extent of the bioreactor cell or infiltration gallery.

- **Regulatory Acceptance.** To date biowall systems have been installed at approximately 13 facilities in 11 states covering five USEPA regions, having overcome all state and federal concerns regarding installation of the biowall systems. Examples of biowalls used for regulatory compliance include the full-scale biowall system installed at Altus AFB, Oklahoma as an interim corrective action; and a biowall system at the Ash Landfill site at Seneca Army Depot Activity, New York that is part of the final remedy in the Record of Decision (ROD) (**Appendix F.1**). As mentioned previously, the Navy was able to transfer the entire NWIRP McGregor property to the City of McGregor in November 2006 with the approval of the Texas Commission on Environmental Quality (TCEQ) and the USEPA.

- **Inexpensive Substrates.** Mulch, compost, and sand are relatively inexpensive when purchased in bulk quantities. Tree mulch can often be obtained for the cost of shipping and handling alone. Amendments to stimulate abiotic processes such as calcium sulfate (gypsum), magnesium sulfate (Epsom salts), or iron sulfate are also common and inexpensive industrial or agricultural products.

- **Low Operation and Maintenance Requirements.** Mulch biowalls require little O&M other than periodic performance monitoring over the first few years of operation. Biowall

systems may need to be replenished with fluid substrates such as emulsified vegetable oil on a periodic basis (perhaps every 3 to 5 years, see **Section 8**), but the cost to replenish is low relative to the capital cost of construction. Bioreactors may also need to be replenished on a periodic basis.

- **Destruction of Contaminants *In Situ:*** Contaminants that are treated have the potential of being completely mineralized or destroyed. Destruction of contaminants *in situ* is highly beneficial because contaminant mass is not transferred to another phase, there is no secondary waste stream to treat, and potential risks related to exposure during remediation are limited.

- **Potential Application to a Variety of Contaminants:** In addition to CAHs, the technology may be applicable to a variety of other contaminants subject to anaerobic degradation processes (**Section 1.4.2**). Multiple contaminants can often be treated simultaneously.

- **Modifications and Contingencies.** Biowall trenches and bioreactors can be modified to include perforated pipe or injection wells (during or after system installation) for addition of fluid substrates to supplement substrate loading, if necessary. Many different configurations are possible. At Altus AFB, Oklahoma for example, groundwater is extracted from a downgradient biowall and recirculated through a source area bioreactor at the SS-17 site.

- **Treatment Train Options:** Biowall and bioreactor systems can be used in tandem, or with existing or alternative remediation systems to optimize performance (*e.g.*, source removal via excavation). They may also be coupled with other *in situ* anaerobic bioremediation techniques. For example, injection of edible oil is being used at Altus AFB, Oklahoma to treat contaminated horizons below the depth of trenching. Alternatively, anaerobic bioremediation systems may be coupled with downgradient aerobic reaction zones (*e.g.*, air sparging trench) to degrade dechlorination products such as *cis*-1,2-DCE or VC that are amenable to degradation by oxidation processes.

Limitations of Permeable Mulch Biowalls

As with many *in situ* treatment technologies, implementation of enhanced *in situ* anaerobic bioremediation causes profound changes to the subsurface environment, and the degree of success may be subject to hydrogeological, geochemical, and biological limitations. Site infrastructure may, in some cases, prevent the installation of biowall trenches. However, some of these limitations also affect other remedial techniques and are not necessarily unique to biowall or bioreactor applications. Several issues that should be considered prior to selecting *in situ* biowalls or bioreactors include, but are not limited to, the following:

- **Site-Specific Limitations.** The depth that can be trenched or excavated in a practical and cost-effective manner is limited to approximately 30 to 35 feet (9 to 10 meters). Benching may be used to reach a greater depth in some cases, but in general the injection of fluid substrates is more economical for depths greater than 35 to 40 feet. In addition, site infrastructure such as utilities may interfere with trenching operations. Other site-specific limitations may be related to difficult geology (*e.g.*, bedrock or large cobbles), hydrogeology (*e.g.*, very high or very low rates of groundwater flow), contaminant distribution (*e.g.*, depth to contamination or presence of dense non-aqueous phase liquid

[DNAPL]), or geochemistry (*e.g.*, adverse pH conditions). Therefore, careful site screening should be conducted prior to selecting the technology (**Section 2.2**).

- **Sustainability.** It is difficult to determine how many years biowall systems will be able to sustain anaerobic degradation processes for chlorinated solvents or other contaminants (*e.g.*, perchlorate) without replenishment of the carbon source. While the mulch fraction may last 10 to 15 years or longer, it may not provide enough readily biodegradable organic carbon to sustain contaminant degradation. Biowalls may need to be replenished with fluid substrates on a periodic basis, perhaps every 3 to 5 years. Fortunately, this is readily accomplished using a suitable O&M plan and proper design.

- **Incomplete Degradation.** Because a single biowall is of finite thickness, the contaminant residence time and the substrate loading rate (*i.e.*, the hydrolysis rate of insoluble organic carbon from mulch that yields smaller and more fermentable dissolved carbon molecules) is limited. Therefore, concentrations of chlorinated solvents in excess of 10 to 100 milligrams per liter (mg/L) may not be completely degraded, resulting in the production and persistence of intermediate dechlorination products. The use of wider trenches or multiple parallel trenches may be necessary to treat higher contaminant concentrations or to deplete high concentrations of native electron acceptors. Alternate configurations allowing for injection of substrate into the biowall to extend a downgradient reaction zone, or for recirculation of groundwater through the biowall system, may also be considered.

- **Secondary Degradation of Water Quality.** While anaerobic dechlorination may be effective in degrading chlorinated solvents and other contaminants, secondary degradation of groundwater quality may occur. Degradation reactions, excess nutrients (*e.g.*, nitrogen and phosphorous), or excessive changes in groundwater pH and reduction-oxidation (redox) conditions may lead to solubilization of metals (*e.g.*, iron, manganese, and potentially arsenic), formation of undesirable fermentation products (*e.g.*, aldehydes and ketones), and other potential impacts to secondary water quality (*e.g.*, total dissolved solids). Stimulating biodegradation also may enhance generation of volatile byproducts and noxious gases (*e.g.*, VC, methane, or hydrogen sulfide) that may degrade groundwater quality and/or accumulate in the vadose zone. Many of these changes are easily reversed when groundwater returns to a more oxidized state downgradient of the treatment zone, but these issues should be considered during technology screening and design.

While these potential limitations should be considered when evaluating enhanced *in situ* anaerobic bioremediation with biowall or bioreactor systems, many of them can be mitigated or compensated for by understanding the hydrogeologic and biogeochemical conditions of the aquifer system (**Section 2.2**) and using an appropriate design (**Section 3**).

1.4.6 Alternative Configurations, Modifications, and Contingencies

Permeable mulch biowall and *in situ* bioreactor systems may be configured in multiple ways. Biowall trenches configured as a biobarrier may be coupled with source area treatment using bioreactors. Multiple trenches or trenches oriented at an angle to groundwater flow may be used to increase residence time (**Section 3.4** through **Section 3.6**). The practitioner should not necessarily limit the application of the technology to conventional configurations where trenches are strictly oriented perpendicular to groundwater flow.

A variety of materials may be used in biowall and bioreactor systems. The local availability of bulk agricultural or landscaping materials *(e.g.*, cotton gin trash or mushroom compost) will often influence selection of biowall materials. Trenches may be modified to include injection wells or perforated pipe for addition of fluid substrates to supplement carbon loading, as necessary. Piping can also be used for recirculation to increase the effective residence time within a biowall or bioreactor treatment system. Recirculation of groundwater may also impact a much larger area of the aquifer than would occur through passive groundwater flow. For example, recirculating groundwater through a bioreactor influences a much greater volume of the aquifer than a passive bioreactor cell due to hydraulic mounding in the bioreactor and more rapid leaching of soluble organic carbon out of the bioreactor cell (see **Figure 1.2**). Similarly, the anaerobic treatment zone may extend downgradient of a biowall trench due to soluble organic carbon migrating out of the biowall with groundwater flow. Finally, the relatively small treatment volume of the trench or bioreactor (relative to other substrate configurations) makes these systems ideal candidates for inoculation with bioaugmentation cultures.

SECTION 2

REMEDIAL OBJECTIVES AND SITE SELECTION

Evaluating remedial objectives and regulatory requirements is the first step in screening a site for application of a permeable mulch biowall or bioreactor. If the technology is a potential remedy for the site, then further site screening is necessary to evaluate technical considerations such as land use and infrastructure, contaminant type(s) and distribution, hydrogeology, geochemistry, and microbiology. This section summarizes remedial objectives and regulatory considerations for biowalls and bioreactors (**Section 2.1**), and reviews site characteristics that are suitable for applying the technology (**Section 2.2**).

2.1 REMEDIAL OBJECTIVES AND REGULATORY REQUIREMENTS

In general, the remedial objective of enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls and *in situ* bioreactors is restoration of groundwater quality to levels protective of human health and the environment. In the case of drinking water aquifers, this is usually to federal- or state-established maximum contaminant levels (MCLs). In some instances, as in the case of emerging contaminants such as perchlorate and RDX for which no Federal MCLs exist, restoration may be to state-mandated cleanup levels. Restoration of contaminated groundwater to pre-existing levels of beneficial use is desirable, but difficult to achieve in practice. In many cases, cleanup criteria may be less stringent if the impacted groundwater does not constitute a potable water supply. Exposure pathways such as surface water discharge also may dictate cleanup criteria. Project- or site-specific remedial objectives may vary accordingly.

Regulatory acceptance of enhanced *in situ* anaerobic bioremediation has evolved over the last decade. Enhanced bioremediation using permeable mulch biowalls has been implemented under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Resource Conservation and Recovery Act (RCRA). The biowall technology has been applied in at least 11 states to date (**Table 1.1**). However, the ability of these biowall systems to meet regulatory guidelines has varied, and many regulatory issues remain regarding 1) the production and persistence of regulated intermediate degradation products, 2) secondary impacts on groundwater quality, and 3) long-term sustainability of biowall performance. But given the proper design, installation, and maintenance of a biowall or bioreactor system, remedial objectives can be met in a cost-effective manner. For example, a series of permeable mulch biowalls were installed at NWIRP McGregor, Texas in 1999 as part of state-approved interim remediation measures for perchlorate in groundwater. The remedy was approved as OPS in June 2006, allowing for transfer of the property to the City of McGregor (CH2M Hill, 2006).

2.1.1 Performance Objectives

Performance objectives for permeable mulch biowall and bioreactor applications are often established by the owner/operator, in addition to drinking water or risk-based cleanup goals that

are often applied by the regulatory community. Examples of performance objectives that biowall systems may be used to meet include the following:

- Reduction of mass discharge from a source zone or across a specified containment boundary.
- Reduction in overall toxicity of the contaminant plume, lessening the potential for completed exposure pathways (Downey *et al.*, 2006).
- Enhancement of already occurring natural attenuation to reduce the duration and cost of long-term monitoring.
- Cost-effective and continuous treatment over relatively long remediation timeframes due to an inability to substantially remediate the contaminant source(s).

Biowall or bioreactor performance objectives based on dissolved contaminant concentrations alone are often difficult to attain over large treatment areas, and may not be representative of the overall reduction in contaminant mass or the reduction in overall toxicity of the plume. For organic contaminants, a significant amount (usually the majority) of contaminant mass in an aquifer system is sorbed to the aquifer matrix (Scow and Johnson, 1997; Grathwohl, 1990; Payne *et al*., 2001). Due to desorption of this contaminant mass, aqueous-phase concentrations alone may not accurately reflect the amount of mass being destroyed if there is continued mass transfer from the sorbed phase to the aqueous phase. For these reasons, the performance of a permeable mulch biowall is often based on reductions in concentrations of contaminants within or immediately downgradient of the treatment system.

2.1.2 Regulatory Requirements

Remedial objectives and performance metrics are driven by regulatory compliance requirements. To design a successful biowall application, the site-specific regulatory framework should be reviewed and compliance standards and remedial endpoints clearly defined. The ability of mulch biowall systems to achieve drinking water MCLs has been demonstrated in some settings, but cannot be assumed to be possible at all sites. The use of less stringent, risk-based remedial goals may be more appropriate and achievable than default drinking water standards.

The potential for production of toxic intermediate degradation byproducts, degradation of secondary drinking water quality, and production of noxious gases should be carefully assessed if potential exposure pathways exist within or immediately downgradient of the biowall or bioreactor treatment system. For example, a typical regulatory concern is the production and persistence of *cis*-1,2-DCE or VC in the reaction zone, which is a result of incomplete sequential dechlorination of PCE or TCE. One factor that is required to achieve adequate degradation of intermediate dechlorination products is an anaerobic reactive zone with sufficient residence time to allow for depletion of the parent compounds with complete sequential dechlorination to innocuous end products. Alternatively, degradation of some compounds such as VC may be accomplished by other degradation processes in a downgradient redox recovery zone (*e.g.*, aerobic environment).

2.2 SITE SCREENING TECHNICAL CONSIDERATIONS

Essentially, the purpose of a permeable mulch biowall or bioreactor is to create an anaerobic treatment zone within the impacted aquifer. Permeable mulch biowalls and bioreactors have been applied at sites with varying stratigraphic, hydrogeologic, and biogeochemical conditions, and can be a cost-effective remedy in many environmental settings. However, there are conditions that may limit the ability to install a biowall or to stimulate complete anaerobic degradation. Therefore, preliminary screening of a site is required prior to selecting a biowall or bioreactor as the remedy of choice. This section describes conditions suitable for implementing biowalls and bioreactors, and those conditions that should trigger consideration of alternative technologies. Additional information and guidance on screening sites for enhanced *in situ* bioremediation may be found in Section 3 of AFCEE *et al.* (2004).

Technical considerations that should evaluated in screening a site for application of enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls or bioreactors may categorized as follows:

- Site infrastructure and use,
- Contaminant type(s) and distribution (*e.g.*, co-contamination or thickness of impacted aquifer),
- Hydrogeology (*e.g.*, depth to groundwater and rate of groundwater flow),
- Groundwater geochemistry,
- Microbiology, and
- Cost effectiveness relative to other remedial alternatives.

Table 2.1 summarizes some common criteria used to determine the suitability of a site for implementing enhanced *in situ* anaerobic bioremediation with permeable mulch biowalls or bioreactors. These are general guidelines only, and there may be notable exceptions to most all of the criteria. For example, bioreactors may mitigate some of the hydrogeological considerations by use of recirculation. Furthermore, while many of these criteria apply to other contaminants such as perchlorate or RDX, suitable site conditions may not be as stringent since these contaminants may degrade under less anaerobic conditions. The criteria listed in **Table 2.1** are discussed in further detail in the following subsections.

2.2.1 Conceptual Site Models

Development of a conceptual site model (CSM) and an understanding of the natural processes that are being stimulated ultimately guides the site selection and system design process. Guidance on developing CSMs and evaluating natural attenuation processes can be found in various publications including USEPA (1998a) and AFCEE *et al.* (2004).

Table 2.1
Suitability of Site Characteristics for the Implementation of Biowalls and Bioreactors

Site Characteristic	Suitable for Biowalls/ Bioreactors	Suitability Uncertain	Suitability Unclear - Possible Red Flag - Requires Further Evaluation
Infrastructure and Land Use	No infrastructure or utilities to interfere with trenching or excavation	Some utilities (*e.g.*, sewer lines) or roadways may be moved or temporarily breached during construction	Presence of buildings or utility lines that cannot be breached, leaving gaps in the biowall or bioreactor
Contaminant Distribution (Depth)	< 35 feet to base of contaminant plume	35 to 45 feet to base of contaminant plume using benching	> 45 feet to base of contamination, beyond practical depth of trenching or excavation
Contaminant Peak Concentrations (CAHs only)	Chlorinated solvent concentrations <10,000 µg/L	Chlorinated solvent concentrations >10,000 µg/L with caution	Mixed contaminant plumes require further evaluation to determine if all contaminants are degraded by anaerobic processes
Evidence of Anaerobic Dechlorination (CAHs only)	Presence of dechlorination products	Limited evidence of anaerobic dechlorination	No evidence of any degradation of CAHs
Lithology	Low permeability, cohesive clay, silt, and silty sand	Weathered or poorly consolidated bedrock	Loose sand and gravel, well consolidated or hard bedrock
Stratigraphy	Biowall extends to a lower confining layer	Lack of a lower confining layer, but where the biowall may extend to the total depth of contamination	Lack of a lower confining layer and uncertainty about the total depth of contamination requires further evaluation
Hydraulic Conductivity	< 1.0 ft/day (< 3 x 10^{-4} cm/sec)	1.0 to 10 ft/day (3 x 10^{-6} to 3 x 10^{-4} cm/sec)	> 10 ft/day (>3 x 10^{-4} cm/sec)
Groundwater Velocity	< 1.0 ft/day	1.0 ft/day to 10 ft/day	> 10 ft/day
pH	6.5 – 7.5	6.0 to 6.5, 7.5 to 8.0	< 6.0, > 8.0
Dissolved Oxygen	< 5.0 mg/L	> 5.0 mg/L combined with groundwater velocity of 1.0 to 10 ft/day	> 5.0 mg/L combined with a high rate of groundwater flow (> 10 ft/day)
Nitrate Concentration (perchlorate)	< 10 mg/L	10 to 20 mg/L combined with groundwater velocity of 1.0 to 10 ft/day	>20 mg/L with caution
Sulfate Concentration (CAHs)	< 1,000 mg/L	1,000 to 5,000 mg/L may be suitable for abiotic degradation processes; High sulfate concentrations have not been observed to inhibit dechlorination of CAHs when using mulch substrates	>5,000 mg/L with caution, may be suitable for abiotic degradation processes

NOTES: CAHs = chlorinated aliphatic hydrocarbons; µg/L = micrograms per liter; mg/L = milligrams per liter; ft/day = feet per day; ft/yr = feet per year; cm/sec = centimeters per second.

With respect to construction and emplacement of solid substrates via trenching or excavation, the presence of underground utilities, rubble or cobbles, flowing or heaving sands, confining layers, cemented sediments (*e.g.*, caliche), and the ability to reach the target depth (with or without benching) should be included in the CSM.

An assessment of degradation potential is primarily based on a review of site-specific data on electron donors, electron acceptors, metabolic byproducts, geochemical indicators, contaminant trends, and hydrogeology. A CSM also summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors. Analysis of contaminant concentration trends can be used to determine whether an ongoing source of contaminant exists at a site, and whether natural attenuation processes are sufficient to control contaminant plume migration. For example, if the aquifer already exhibits reducing conditions while target contaminant concentrations simultaneously persist, then the application of an active or passive organic substrate addition technology may not be productive. Such conditions might be indicative of high contaminant discharge that exceeds the rate at which the contaminants can be degraded by anaerobic processes.

2.2.2 Land Use and Infrastructure

Biowall trenches or bioreactor excavations may interfere with site infrastructure. Installation of a trench or excavation is not feasible underneath or immediately adjacent to buildings. Some utilities may be temporarily breached during installation (*e.g.*, storm sewer lines), but others (*e.g.*, gas lines or communication lines) may not be practical or may be cost prohibitive to breach. Overhead electrical lines must also be avoided for safety considerations during construction.

In the event a utility cannot be breached during construction (*e.g.*, fiber optic communication lines or high pressure gas lines), the continuity of the biowall or bioreactor reactive zone may be maintained by injection of slow-release substrates (*e.g.*, emulsified vegetable oil) in groundwater below the utility. But in general, installation of a biowall or bioreactor is best suited for open areas with few utilities.

For bioreactor or infiltration trenches using recirculation of groundwater, a local source of electrical power may be necessary. In remote locations without access to a power supply, solar powered pumps may be utilized. An example of a solar powered recirculation system for the bioreactor at LF-03, Altus AFB, Oklahoma is shown in **Figure 2.1**, and described in **Appendix F.3**.

2.2.3 Contaminant Distribution and Peak Concentrations

The vertical extent of contamination is a primary consideration in applying permeable mulch biowalls due to limitations in the depth that can be trenched. The biowall system must be able to intercept the plume without unacceptable contaminant bypass either below or around the biowall system. In some cases, monitored natural attenuation (MNA) may be sufficient as a remedy for low concentrations of contaminants that bypass a biowall trench. Alternatively, deeper injection wells below the biowall trench could be employed where only limited portions of the contaminant plume are not intercepted by the biowall trench. Biobarriers constructed by direct injection of slow release substrates are likely to be more economical where large portions of the contaminant plume migrate below the limits of trenching. Similar consideration of the depth of contamination should be applied for construction of bioreactor systems.

Figure 2.1 Solar Panel to Power an Extraction Pump for the Remote Bioreactor Site at LF-03, Altus AFB, Oklahoma

Enhanced anaerobic bioremediation takes advantage of natural processes that may already be contributing to the degradation of the contaminants present. For example, the presence of intermediate dechlorination products that indicate that biotic reductive dechlorination of CAHs is occurring or has occurred naturally is a favorable indicator. Conversely, the lack of any dechlorination products is a "red flag" that biotic reductive dechlorination may be difficult to stimulate and that further evaluation is warranted (*e.g.*, biogeochemical screening, column studies, or pilot testing). Note that alternate anaerobic degradation processes for CAHs such as anaerobic oxidation or biogeochemical transformation do not produce intermediate dechlorination products (**Section B.4** in **Appendix B**), and the potential for these processes to occur should also be evaluated during the screening process for CAHs.

Because biowall trenches are typically less than 2 to 3 feet in thickness, the residence time of the contaminant within the biowall reaction zone may not be sufficient at sites with high contaminant concentrations and/or high groundwater velocities. Note that a reactive treatment zone is typically created directly downgradient of the biowall where additional treatment and polishing occurs. Remedial approaches using biowalls should consider downgradient anaerobic and aerobic degradation processes in addition to residence time within the biowall proper. Multiple biowall sections may be required to treat concentrations of CAHs in excess of 10,000 micrograms per liter (µg/L) depending on the rate of groundwater flow. Concentrations of perchlorate up to 20,000 µg/L have been treated with single biowalls at NWIRP McGregor, but the ability to treat perchlorate concentrations above 20,000 µg/L may require more aggressive designs. Bioreactors that recirculate groundwater may be able to treat much higher contaminant concentrations because recirculation increases the effective residence time of the contaminant in the reaction zone.

For other contaminants subject to anaerobic degradation processes (*e.g.*, RDX and TNT) or for mixed contaminant plumes, the residence time should be based on the contaminant that will take the longest to degrade to remedial objectives. Biowalls or bioreactors can handle mixed contaminants as long as they are anaerobically degradable and the reaction kinetics are

sufficiently understood to design a system that can degrade them to applicable remedial objectives. This will be a function of both contaminant concentration and degradation rate for each contaminant type. The rate of degradation for contaminants such as RDX or TNT that is observed in other enhanced anaerobic bioremediation applications may offer insight into the concentrations that can be treated using a permeable mulch biowall or bioreactor. Degradation rates versus residence time in the reaction zone are discussed in greater detail in **Section 3.4**.

2.2.4 Hydrogeology

The uncertainty in characterizing subsurface hydrogeology complicates all *in situ* treatment technologies, and must be considered during the site selection and design process. Inadequate characterization of the site hydrogeology can lead to failure of the remedy to meet performance and regulatory objectives. Difficult hydrogeologic conditions that may preclude cost-effective application of biowalls or bioreactors include high rates of groundwater flow, high permeability, high levels of aquifer heterogeneity, presence of preferential flow paths, or excessive depth to groundwater. Additional characterization of hydrogeologic conditions may be warranted if the site is not well characterized.

2.2.4.1 Lithology

Installation of biowall trenches or bioreactor excavations may be limited by 1) subsurface formations that are too hard or consolidated (*e.g.*, competent bedrock) for trenching or excavation by available construction equipment, or 2) sediments that are too unconsolidated (*i.e.*, flowing sands) to remain open while the substrate mixture is being emplaced. Some weathered, poorly cemented, or poorly indurated bedrock formations may be cut by trenchers with special rock cutting teeth. For example, **Figure 2.2** illustrates a special trenching machine used to excavate into soft limestone bedrock at NWIRP McGregor. The trenching contractor should be consulted to determine the ability to trench into bedrock materials. Blow count data from split spoon sampling is one measure of relative resistance to trenching that is useful to provide to the construction contractor.

In unconsolidated sediments, it may be difficult to keep the trench open long enough to emplace the biowall backfill materials. Loose or flowing sands will make biowall installation difficult or impractical. Large cobbles or boulders may also inhibit the ability to trench the formation. In addition, the presence of permeable, high-yielding aquifer materials may result in a water-filled trench, making placement of the mulch and sand mixture problematic. These materials have significantly different densities and will tend to separate when passed through a standing column of water. Note that continuous trenchers have a loading chute and an excavation trench box behind the cutting teeth of the trencher (**Figure 1.1**). Under favorable conditions the mulch/sand media is continuously delivered to depth and a standing column of water does not accumulate.

Figure 2.2 Rock Trencher Employed at NWIRP McGregor, Texas
(Photo Courtesy of US Navy)

2.2.4.2 Groundwater Hydraulics

Depth to Groundwater and Depth of Contamination. Depth to water and the depth of contamination also determine whether a biowall or bioreactor system can be installed. There are practical limits to the depth that can be trenched or excavated, typically 30 to 35 feet in moderately cohesive sediments. It is preferable that the biowall trench be installed to a lower confining layer. In the event a confining layer is not present, the biowall trench should extend at least to the total depth of significant contamination to prevent bypass beneath the biowall.

Groundwater Flow. Groundwater velocity and flow direction will impact the effectiveness of a biowall or bioreactor application. Groundwater seepage velocity and flow direction are determined by measuring both horizontal and vertical hydraulic gradients, as well as hydraulic conductivity (K). Horizontal groundwater flow rates impact the residence time of the contaminants within the biowall treatment zone. High rates of groundwater flow reduce contaminant residence time in the biowall or bioreactor, while low rates of groundwater flow increase the effective residence time. Where the biowall is installed to a low permeability confining layer, vertical gradients and flow will generally not be an issue.

The highest potential seepage velocity or specific discharge (*i.e.*, Darcy velocity resulting from the highest K and gradient) that may be encountered at a site should be used for site screening and system design. While it may not be practical to determine the absolute highest rate of groundwater flow that may occur within high permeability sediments at the site, an estimate of an upper bound for hydraulic conductivity can be made based on aquifer test results for high permeability zones or from literature values for similar sediments. A reasonable upper bound to hydraulic conductivity can be used to estimate conservative groundwater flow rates for screening and design purposes.

Groundwater seepage velocities of less than 1 foot per day (ft/day), or 360 feet per year (ft/yr), are generally suitable for biowall systems, while seepage velocities greater than 1 ft/day (360 ft/yr) will likely require multiple biowall trenches to effectively remediate the contaminant plume. The magnitude of the groundwater seepage velocity or specific discharge dictates how robust the system should be. Often dual biowalls or multiple sets of biowalls spaced along the axis of the plume are required to provide greater net residence time of contaminants in the reactive treatment zone. Contaminants that have a relatively high degradation rate without production of persistent regulated intermediate products (*e.g.*, perchlorate) may require significantly less residence time than chlorinated solvents.

In addition, very low rates of groundwater flow may not be suitable when a measurable impact on downgradient water quality is desired in a relatively short period of time. For example, at a groundwater seepage velocity of 10 ft/yr, it might take 10 years to see an impact on water quality at a point of compliance located 100 feet or more downgradient of a biowall or bioreactor. In this case a recirculating system may be useful to increase the volume of groundwater that can be treated.

Hydraulic Conductivity and Heterogeneity. Hydraulic conductivity is an indicator of the ability of groundwater to flow through the formation, and is directly proportional to the rate of groundwater flow. In general, biowalls or bioreactors may be used for a broad range of hydraulic conductivity if the rate of groundwater flow (*i.e.*, hydraulic gradient) is not excessive. It is necessary to construct a biowall with higher hydraulic conductivity than the surrounding formation, otherwise groundwater flow will tend to flow around the biowall. Flow through the biowall can also be encouraged by using hydraulic controls and keying the biowall into a lower confining layer or bedrock. There is likely a limit to the hydraulic conductivity (perhaps 10 to 100 ft/day) where the formation is sufficiently cohesive to install a biowall trench or bioreactor excavation.

It is recommended that the practitioner have a clear understanding of the subsurface vertical profile with respect to contaminant distribution, soil layers with relatively high or low hydraulic conductivity, the presence of confining layers, and the potential for vertical gradients. There may be sites where contaminants are primarily localized or sorbed in lower permeability soils where more discrete groundwater sampling and profiling of hydraulic conductivity would significantly alter an evaluation of biowall feasibility and design. The significance of preferential flow paths can only be evaluated and factored into a design if the vertical profiling of contaminants is conducted on scale commensurate with the thickness and distribution of individual soil layers that exhibit variation in hydraulic conductivity. Formations with secondary permeability such as fractures or weathered horizons require special consideration as to how groundwater flows through the aquifer. The existence of high conductivity layers may be evaluated through careful logging of soil cores, sampling and analysis of contaminant concentrations in discrete soil horizons, borehole flow meter testing, and/or aquifer testing of discrete horizons to determine variation in hydraulic conductivity.

The presence of heterogeneity in the formation infers the presence of preferential flow paths. This may result in channeling of groundwater and dissolved contaminants through the biowall, although the homogeneity of the biowall backfill will cause some mixing of groundwater in the trench. ***Most importantly, the biowall system must be designed to account for preferential flow paths, and the design should consider the highest potential rate of groundwater flow through the biowall trench.***

2.2.5 Groundwater Geochemistry

The addition of an organic substrate to an aquifer is intended to consume native electron acceptors and maintain optimal conditions for high rates of anaerobic degradation to occur. Excessive levels of native electron acceptors (*e.g.*, dissolved oxygen [DO], nitrate, bioavailable iron, and sulfate) may limit the ability to achieve sufficiently reducing conditions for effective and complete anaerobic degradation. Due to the bulk and reducing capacity of mulch and compost substrates commonly used in biowalls and bioreactors, it is rare that the native electron acceptor demand cannot be overcome. However, groundwater geochemical characteristics across the site should be reviewed to identify any limiting conditions that would indicate the need for a more robust design.

Dissolved Oxygen and Oxidation-Reduction Potential. Background levels of DO and values of oxidation-reduction potential (ORP) are indicators of the natural redox conditions that must be lowered or optimized within the reactive zone to achieve efficient anaerobic degradation. In general, concentrations of DO less than 0.5 mg/L and ORP levels less than 0 millivolts (mV - relative to a standard hydrogen electrode [SHE]) are desired to stimulate anaerobic degradation processes. Elevated levels of DO in most aquifers can be overcome by providing adequate amounts of substrate. However, the problem may be compounded by other factors such as a high rate of groundwater flow. Concentrations of DO greater than 5 mg/L combined with a groundwater seepage velocity greater than 1 ft/day requires careful consideration to ensure a biowall or bioreactor design will achieve the reducing conditions necessary for effective degradation of the contaminant of concern.

For example, little or no degradation of TCE was observed within a biowall installed at the BG-05 site at Ellsworth AFB, South Dakota (Parsons, 2006c). Background concentrations of DO (6.3 to 7.5 mg/L) and sulfate (440 to 590 mg/L), combined with groundwater seepage velocities estimated to range from 200 to 1,000 ft/yr, appear to limit the formation of anaerobic conditions within the mulch biowall. Even though DO was reduced to less than 2.0 mg/L in the biowall, only very limited sulfate reduction and degradation of TCE to *cis*-1,2-DCE was observed. In this case, additional biowalls or injection of supplemental organic carbon is likely required to stimulate reducing conditions sufficient for anaerobic dechlorination of TCE.

Nitrate. Nitrate is a native electron acceptor that may be found in agricultural or heavily landscaped areas (*e.g.*, golf courses) Similar to DO, nitrate less than 10 to 20 mg/L can be readily overcome in most aquifers by providing adequate substrate. Special consideration for substrate composition or replenishment options are warranted for perchlorate applications in cases where nitrate is greater than 10 to 20 mg/L.

Sulfate/Sulfides. Existing guidance documents tend to suggest that, while CAH dechlorination under sulfate-reducing conditions is feasible, high sulfate levels may be problematic for complete and effective CAH bioremediation. The anaerobic dechlorination scoring matrix in the USEPA (1998a) protocol results in a lower score (lower potential for anaerobic dechlorination) if sulfate exceeds 20 mg/L; similar cautions are provided by Morse *et al*. (1998). However, there is ample evidence for dechlorination of a variety of CAHs at sites containing elevated levels of dissolved sulfate (*e.g.*, **Appendix F.2** and **Appendix F.3**). Complete anaerobic dechlorination has been stimulated at several high-sulfate Air Force sites including Altus AFB, Oklahoma (sulfate up to 2,600 mg/L) and Travis AFB, California (sulfate up to 5,400 mg/L). Therefore, the presence of high sulfate concentrations does not preclude application of this technology. In fact, ***high sulfate sites are candidates for stimulating***

biogeochemical transformation of CAHs through the formation of reactive metal sulfides. A description of this process is provided in **Section B.4.4** in **Appendix B**.

pH and Alkalinity. A pH close to neutral (*i.e.*, 6.5 to 7.5) is the most conducive to the proliferation of healthy, diverse microbial populations. Low pH conditions (<6.0) are detrimental to sulfate-reducing, methanogenic, and dechlorinating bacteria. Fermentative organisms favor lower pH conditions, and therefore will out-compete sulfate-reducing and methanogenic bacteria in more acidic environments; this can result in the formation of undesirable byproducts of fermentation such as ketones, alcohols, and aldehydes. In addition, the abiotic reaction of CAHs with FeS has been reported to increase ten-fold with each unit increase in pH (Butler and Hayes, 2001), and maintaining or increasing pH within the biowall may be beneficial if biogeochemical transformation of CAHs is targeted as a primary degradation process. Alternatively, acidogenic fermenters (which tend to produce molecular hydrogen) might be ideal organisms for achieving the reduction of perchlorate and explosive compounds. The optimal pH of such organisms is likely to be a pH unit or more lower than neutral.

Aquifer systems with lower buffering capacities are more susceptible to decreases in pH. Alkalinity is a general indicator of buffering capacity. However, because of the importance of the aquifer solids in providing buffering capacity, groundwater alkalinity may underestimate the true buffering capacity. Commercial analytical methods are available to measure the buffering capacity of native soils. From a practical standpoint, natural alkalinity greater than 300 mg/L is generally sufficient to buffer against adverse pH changes. Alkalinity less than 300 mg/L warrants further consideration of aquifer buffering capacity.

The addition of a buffering material should be considered at sites with low alkalinity (less than 300 mg/L) or acidic pH (less than 6.5). If the buffering capacity of the formation and biowall materials is in doubt, crushed limestone or recycled concrete may be used during construction to neutralize and/or raise pH. It is more practical to add a solid-phase buffering agent during construction than attempt to add a buffering agent post construction through injection of a fluid amendment.

2.2.6 Soil Chemistry and Mineralogy

Soil chemistry and mineralogy are often overlooked in many enhanced *in situ* bioremediation applications. The bioavailability of common electron acceptors such as ferric iron (Fe^{3+}) and manganese (Mn^{4+}) often cannot be determined from conventional groundwater monitoring protocols. While reduction of high levels of bioavailable ferric iron (as iron oxide or iron hydroxide minerals) or manganese (as manganese oxide minerals) may utilize a large proportion of organic substrate, the amount of reducing capacity provided by mulch and compost substrates is almost always sufficient to overcome the electron acceptor demand. More importantly, the amount of bioavailable iron present in native soil or backfill material may indicate the potential for stimulating the production of reactive iron sulfide minerals and the consequent abiotic dechlorination of CAHs. Therefore, it may be useful to analyze for bioavailable iron if soils or backfill material with high iron are expected, or desired. Soil analytical protocols for iron and sulfides are described in **Section 6,** and a description of anaerobic degradation of CAHs by iron sulfides can be found in **4.4** of **Appendix B**.

2.2.7 Microbiology

Enhanced anaerobic bioremediation of CAHs is typically intended to stimulate microbially mediated anaerobic reductive dechlorination. The success of the technology largely depends on the presence of appropriate dechlorinating bacteria and the ability to stimulate sufficient growth and activity to degrade contaminants to the extent and at a rate that meets the intended remedial objectives. Incomplete dechlorination may lead to accumulation of intermediate dechlorination products such as *cis*-1,2-DCE or VC due to insufficiently reducing conditions or lack of appropriate dechlorinating populations. Alternatively, complete degradation may be achieved by other degradation processes including biogeochemical transformation or anaerobic oxidation.

Determining the potential for complete anaerobic degradation of CAHs may be difficult during the site screening process. Without evidence of even limited degradation (*e.g.*, no degradation past *cis*-1,2-DCE), confidence in the potential to stimulate complete dechlorination by biostimulation alone is unknown. It may be appropriate to simply observe whether biogeochemical conditions for stimulating anaerobic dechlorination of CAHs can be induced in a bench or pilot test, and then to sample and analyze for appropriate dechlorinating populations.

Microorganisms for reduction of perchlorate and explosive compounds appear to be ubiquitous in the environment (*e.g.*, Logan, 1998; Logan *et al.* , 1999; Xu *et al.* , 2003; Ederer *et al*., 1993; Regan and Crawford, 1994; Zang and Hughes, 2002; Ahmad and Hughes, 2000 and 2002) and microbial characterization is usually not warranted. Further discussion of anaerobic biodegradation of perchlorate and explosive compounds is included in **Appendix B**.

2.2.8 Secondary Water Quality

Several changes in water quality may occur during anaerobic bioremediation. These changes occur primarily within the anaerobic treatment zone and may be of concern if drinking water aquifers are present and primary/secondary drinking water standards are applicable. The term "secondary water quality" is used in this document to refer to water quality issues or concerns, apart from the primary contaminants being treated, that result from substrate addition.

Degradation of secondary water quality can occur as a result of elevated levels of organic carbon and nutrients (*e.g.*, nitrogen and phosphorous) released to the aquifer and the stimulation of anaerobic processes. Secondary water quality parameters that may be impacted include chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS), sulfides that affect taste and odor, and discoloration of groundwater from soluble iron. These parameters should be monitored if regulated at the site. **Table 2.2** lists some of the common parameters monitored during enhanced bioremediation and associated federal water quality standards. This list is not exhaustive, as many states enforce additional water quality standards.

In general, the reduced groundwater environment induced by construction of a biowall or bioreactor may increase the mobility of some naturally occurring (but regulated) metals in the reactive zone (*e.g.*, iron, manganese, and arsenic). Migration of metals such as arsenic may be retarded by sorption to the aquifer matrix. More notably, the mobilized inorganics are typically precipitated/immobilized downgradient of the reactive zone when the groundwater conditions return to a more oxidizing state closer to background conditions.

Table 2.2
Water Quality Parameters Subject to Regulatory Compliance
at Enhanced Anaerobic Bioremediation Sites

Compound or Element	Molecular Formula	USEPA MCL (mg/L)[a/]	USEPA Secondary Standard [b/] (mg/L)
Chloroethenes			
Tetrachloroethene (PCE)	C_2Cl_4	0.005	--
Trichloroethene (TCE)	$C_2 HCl_3$	0.005	--
cis-1,2-Dichloroethene (*cis*-1,2-DCE)	$C_2 H_2Cl_2$	0.070	--
trans-1,2-Dichloroethene (*trans*-1,2-DCE)	$C_2 H_2Cl_2$	0.100	--
1,1-Dichloroethene (1,1-DCE)	$C_2 H_2Cl_2$	0.007	--
Vinyl chloride (VC)	C_2H_3Cl	0.002	--
Chloroethanes			
1,1,1-Trichloroethane (1,1,1-TCA)	$C_2H_3Cl_3$	0.200	--
1,1,2-Trichloroethane (1,1,2-TCA)	$C_2H_3Cl_3$	0.005	--
1,2-Dichloroethane (1,2-DCA)	$C_2H_4Cl_2$	0.005	--
Chloroethane (CA)	C_2H_5Cl	0.0046 [e/]	--
Chloromethanes			
Carbon tetrachloride (CT)	CCl_4	0.005	--
Chloroform (CF)	$CHCl_3$	0.1 [c/]	--
Dichloromethane (DCM) (or methylene chloride)	CH_2Cl_2	0.005	--
Total Trihalomethanes (includes CF)	--	0.080	--
Perchlorate			
Perchlorate	ClO_4^-	--	24.5
Chlorite [d/]	ClO_2^-	1.0	--
Chloride [d/]	Cl^-	--	250
Explosive Compounds			
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	$C_3H_6N_6O_6$	0.00061 [e/]	--
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	$C_4H_8N_8O_8$	1.8 [e/]	--
2,4,6-Trinitrotoluene (TNT)	$C_7H_5N_3O_6$	0.0022 [e/]	--
Inorganics			
Arsenic [d/]	As	0.010	--
Selenium [d/]	Se	0.05	--
Iron [d/]	Fe	--	0.3
Manganese [d/]	Mn	--	0.05
General Water Quality Parameters			
Nitrate (as nitrogen)	NO_3^-	10	--
Nitrite (as nitrogen) [d/]	NO_2^-	1.0	--
Sulfate	SO_4^-	'--	250
pH	--	--	<6.5, >8.5
Total dissolved solids (TDS) [d/]	--	--	500
Odor	--	--	3 threshold odor number
Color	--	--	15 color units

[a/] USEPA MCL = USEPA Maximum Contaminant Level; mg/L = milligrams per liter.

[b/] National secondary drinking water regulations are non-enforceable guidelines. However, states may choose to adopt them as enforceable standards.

[c/] Tentative MCL (pending).

[d/] These are compounds or elements that in some cases may increase in concentration as the result of anaerobic bioremediation.

[e/] USEPA drinking water MCL for this compound does not exist, concentration is based on the USEPA Region 9 Preliminary Remediation Goal (PRG) for tap water.

Stimulating biodegradation also may enhance generation of volatile byproducts and noxious gases (*e.g.*, VC, methane, and hydrogen sulfide) that may degrade groundwater quality and/or accumulate in the vadose zone. In addition, these gases can accumulate within subsurface structures (*e.g.*, basements, utility corridors) in the immediate vicinity of a treatment zone. Evaluation of the potential for gas generation can be performed during engineering design of an individual system. Factors to be considered include depth to the zone of interest, potential concentrations and volumes of gases that may be produced, potential pathways for vapor migration, proximity of structures and underground utility corridors, and potential receptors such as building occupants.

Passive diffusion of these gases to the atmosphere and *in situ* degradation during transport may be sufficient to mitigate any safety concerns. However, vapor-phase concentrations of these compounds should be monitored when a potential concern exists to ensure that safe conditions are maintained. Monitoring of potentially explosive gases should be considered for public safety.

2.2.9 Reviewing Field Data for Anaerobic Biodegradation Potential

The primary objective of permeable mulch biowall and bioreactor applications is to stimulate anaerobic degradation of contaminants to levels protective of human health and the environment. Because anaerobic dechlorination occurs sequentially, both the parent CAHs and their dechlorination products must be degraded to protective levels. Therefore, it is important to evaluate the potential for complete dechlorination of CAHs to innocuous end products to occur. It also may be beneficial to evaluate the potential for other degradation processes to achieve the same end result, such as biogeochemical transformation, anaerobic oxidation, or aerobic oxidation of VC or chloroethane (CA) in a downgradient redox recovery zone.

Evaluating the potential to stimulate anaerobic dechlorination at a site has much in common with evaluating natural attenuation processes. Both assessments are based on multiple converging lines of evidence that include a review of degradation byproducts, contaminant trends, electron donors, electron acceptors, metabolic byproducts, geochemical indicator parameters, and hydrogeology. However, evaluating the potential for enhanced anaerobic bioremediation requires extrapolating current site conditions to predict the impact of adding large quantities of organic substrate to the aquifer. Additional discussion of determining the potential of enhanced anaerobic bioremediation can be found in Sections 3 and 4 of AFCEE *et al.* (2004), including development of CSMs and pre-design techniques (such as specialized analytical methods and bench scale tests) that may be used to better assess whether enhanced *in situ* bioremediation will stimulate complete anaerobic dechlorination of CAHs.

2.3 PROCEEDING WITH BIOWALL SYSTEM DESIGN AND INSTALLATION

If preliminary screening for enhanced bioremediation using a permeable mulch biowall or bioreactor system indicates that it is a useful and appropriate remedial strategy, the practitioner or environmental manager should also consider whether it is the most reasonable approach. This should include a cost comparison to alternative technologies such as MNA, groundwater extraction and treatment, air sparging, chemical treatment, zero-valent iron walls, or other enhanced anaerobic bioremediation approaches.

The ability to evaluate the potential for a biowall or bioreactor system to meet remedial objectives during the preliminary screening process is dependent on adequate site characterization. ***Inadequate site characterization is the most common cause of the failure of enhanced anaerobic bioremediation systems to meet regulatory criteria. Further site characterization and evaluation may lower the risk that a biowall or bioreactor design is inadequate for site conditions.*** In many cases, the use of more robust designs (multiple biowalls) and incorporation of contingency measures (injection piping for rejuvenation) can mitigate unexpected site conditions such as the presence of preferential flow paths or areas of high rates of groundwater flow.

There are a number of system and engineering design options for applying enhanced anaerobic bioremediation via a biowall or bioreactor. Selection of a practical technical approach and system configuration should be based on meeting site-specific remedial objectives. Once remedial goals and a suitable technical approach (*e.g.*, source reduction or biobarrier containment) are established, the practitioner faces a multitude of design considerations. **Section 3** describes the technical approach and engineering considerations that are used to design effective biowall and bioreactor systems.

SECTION 3

SYSTEM DESIGN AND ENGINEERING

3.1 TECHNICAL APPROACH

Design of a biowall or bioreactor system revolves around remedial objectives and site-specific conditions, including an appropriate configuration to intercept and treat the contaminant plume. The design of permeable mulch biowalls and *in situ* bioreactors requires consideration of the following:

- Configuration as a biowall to intercept and attenuate solute plumes or to reduce contaminant discharge at key locations, or as an *in situ* bioreactor to treat residual source areas such as landfill excavations (**Section 3.2**).
- Site-specific hydrogeology and contaminant distribution (**Section 3.3**).
- Dimensions of the treatment zone in relationship to degradation rates, contaminant concentrations, and residence time (**Section 3.4** through **Section 3.6**).
- Selection of suitable materials for biowall or bioreactor construction (**Section 3.7**).

Groundwater flow rates and the residence time in the reaction zone are two primary design considerations for a biowall or bioreactor configuration.

Biowall systems should be conservatively designed based on achievable degradation rates, and use a conservative groundwater flow rate based on the degree of aquifer heterogeneity and presence of preferential flow paths.

- Installation and construction methods (**Section 3.8**).
- Modifications and contingencies, such as piping for replenishing with fluid substrates or recirculation of groundwater (**Section 3.9**).
- Regulatory concerns, including disposal of trenching spoils (**Section 3.10**).
- Monitoring network configuration (**Section 3.11**).

Groundwater flow rates, degradation rate(s), and the residence time of the contaminant(s) in the biowall or bioreactor reaction zone are the primary design considerations in determining the system configuration and dimensions. Materials must be selected and procured, and any modifications or contingencies for optimization of the system should be incorporated into the

design prior to installation. The following sections describe the rationale for development of a system design based on the considerations outlined above.

3.2 SYSTEM CONFIGURATIONS

Biowalls using solid mulch and compost substrates are constructed in a trench in a permeable biobarrier configuration (**Section 3.2.1**). Other variations of using mulch and compost substrates in flow-through configuration include bioreactors constructed by burial of mulch in excavations (**Section 3.2.2**). Mulch applications by burial are limited by the depth to which the substrate can be placed, and therefore are generally suitable only for relatively shallow groundwater plumes. Surface amendments that rely on infiltration of precipitation have also been applied, for example at Offutt AFB, Nebraska (Groundwater Services, Inc. [GSI], 2001).

Groundwater recirculation (**Section 3.2.3**) may be used in either a biowall or bioreactor configuration to 1) increase the effective residence time of the contaminant in the treatment zone, 2) capture groundwater from a greater volume of the aquifer, and/or 3) expand the treatment zone in either a vertical or horizontal direction. O&M requirements will be greater for recirculation systems.

3.2.1 Biowall Trenches

Biowall configurations rely on the flow of groundwater under a natural hydraulic gradient through the biowall to promote contact with slowly soluble organic matter. This configuration is particularly suitable for low permeability or highly heterogeneous formations, as the formation is physically removed and the biowall trench effectively exposes the contaminant plume to the substrate fill material. The continuity of the trench reduces potential problems of groundwater bypass resulting from preferential flow paths.

Many demonstration projects have used single biowalls oriented perpendicular to groundwater flow. Other configurations may be used to increase the effective residence time of contaminants in the reaction zone. Multiple biowalls in parallel may be used, or biowalls may be oriented at an angle to groundwater flow to induce a component of flow along the biowall trench. **Figure 3.1** illustrates a dual biowall configuration installed at Dover AFB, Delaware. The biowalls were angled towards the center of the plume to prevent potential bypass around the ends of the biowalls.

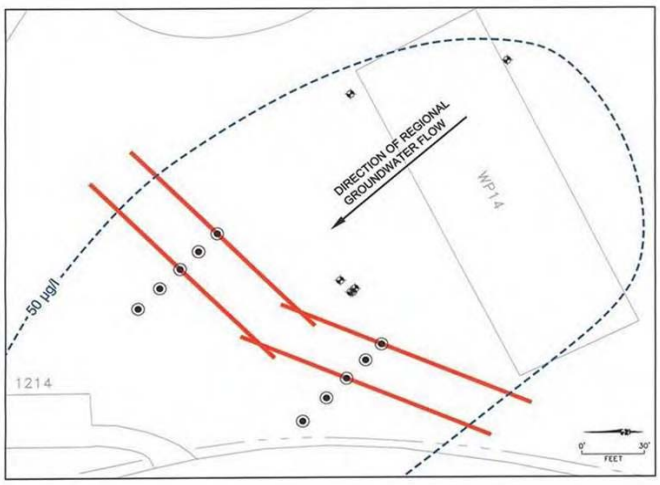

Figure 3.1 Dual Biowall Configuration at Dover AFB, Delaware

Trenches may be installed using either continuous one-pass trenchers or hydraulic excavators, which are basically backhoes with extended booms. Trench depths are limited by the type of equipment used, the stability of the formation, and the ability of the equipment to excavate the formation. Biowall dimensions are based on site-specific conditions discussed in **Section 3.4** and **Section 3.5**.

3.2.2 Bioreactors and Surface Amendments

Another useful application for mulch and compost is to line landfill or source area excavations with a mulch mixture to form a bioreactor (**Figure 1.2**). Inclusion of a bark-mulch sub-layer in alternative landfill covers also may be considered. Mulch or compost can be placed in excavations below the water table, but mulch placed above the water table relies on natural or enhanced infiltration (*e.g.*, via recirculation of captured groundwater) to be effective.

Surface amendments can be constructed by placing several feet of a mulch or compost on the ground surface or within a shallow excavation (GSI, 2001; Haas *et al.*, 2000). Amendments that rely on precipitation and natural infiltration to leach organic carbon into shallow contaminated groundwater require a favorable water balance between precipitation, evapotranspiration, and infiltration. Climatic conditions will factor strongly into site selection for surface amendments.

3.2.3 Recirculation Configurations

While permeable biowalls are designed primarily as passive flow-through barriers, they also may be modified to capture and extract groundwater for recirculation. Recirculation can be incorporated into a biowall design by adding piping or extraction wells. Continuous one-pass trenchers are designed for de-watering of groundwater, where a 3- to 4-inch perforated high density polyethylene (HDPE) pipe (or similar material) is installed at the base of the trench excavation. This pipe can be incorporated into the recirculation design for either extraction or injection.

Recirculation may be incorporated into a bioreactor by installation of extraction wells beneath or downgradient of the bioreactor cell, or by installation of an extraction trench downgradient of the bioreactor cell. Recirculation is an effective way to expand the reaction zone beneath and adjacent to a bioreactor. Groundwater pumped into the bioreactor cell forms a groundwater mound, forcing water to migrate both vertically and horizontally out from the bioreactor cell. Because this water contains soluble organic carbon that leaches from the mulch or compost mixture, the effective treatment zone is expanded by several times the volume of the bioreactor cell itself (**Appendix F.3**).

3.3 HYDROGEOLOGY AND CONTAMINANT DISTRIBUTION

Biowall design criteria are based on the characteristics of the subsurface and the contaminant plume, including subsurface lithology, groundwater hydraulics, groundwater geochemistry, and the nature and extent of contamination.

The geology of the subsurface and groundwater hydraulics are primary considerations in biowall designs. Contaminant residence time is dependent on the rate of groundwater flow. The presence of preferential flow paths with high rates of groundwater flow may adversely impact the ability of the biowall system to meet remedial objectives. Thus, biowall systems should be conservatively designed to treat the highest potential rates of groundwater flow.

Impacts to hydraulic conductivity during enhanced bioremediation can be attributed to the following (GeoSyntec, 2005):

- Biological fouling (biofouling) of the aquifer due to biomass growth, and

- Gas clogging from excessive amounts of dissolved gases including carbon dioxide, methane, and hydrogen sulfide.

A reduction in hydraulic conductivity due to biomass growth or generation of biogenic gases may potentially lead to preferential flow of groundwater around a biowall trench or bioreactor cell. However, significant reductions in hydraulic conductivity were not observed or documented in the case studies reviewed during the preparation of this document. It is not anticipated that biomass growth or generation of biogenic gases will negatively impact hydraulic conductivity for typical biowall or bioreactor applications where the permeability of the biowall or bioreactor matrix is an order of magnitude or more greater than the surrounding formation.

The vertical extent of contamination is a primary consideration in applying permeable mulch biowalls due to limitations in the depth that can be trenched. The biowall system must be able to intercept the plume without unacceptable contaminant bypass either below or around the biowall trenched. Bioreactors using recirculation to expand the volume of aquifer that is captured and treated, or to expand the volume of aquifer impacted by soluble organic carbon, should also be deigned to capture the vertical and horizontal extent of the impacted aquifer. The following sections describe the criteria for determining the appropriate dimensions of a biowall or bioreactor system.

3.4 BIOWALL DIMENSIONS

The dimensions of the biowall sections are critical to achieving performance objectives and the overall success of the remedy. Biowall dimensions used in this protocol are shown on **Figure 3.2**, and include length (x), thickness or width (y), and depth (z). Note that the reaction zone may extend downgradient of the biowall trench due to the release of soluble organic carbon from biowall materials and migration with groundwater flow. Multiple biowalls may be installed as a "system" to achieve a sufficient reaction zone. A continuous reaction zone may be achieved by spacing multiple biowalls close enough that soluble organic carbon migrating from one biowall sustains an anaerobic reaction zone within the formation to the next downgradient biowall.

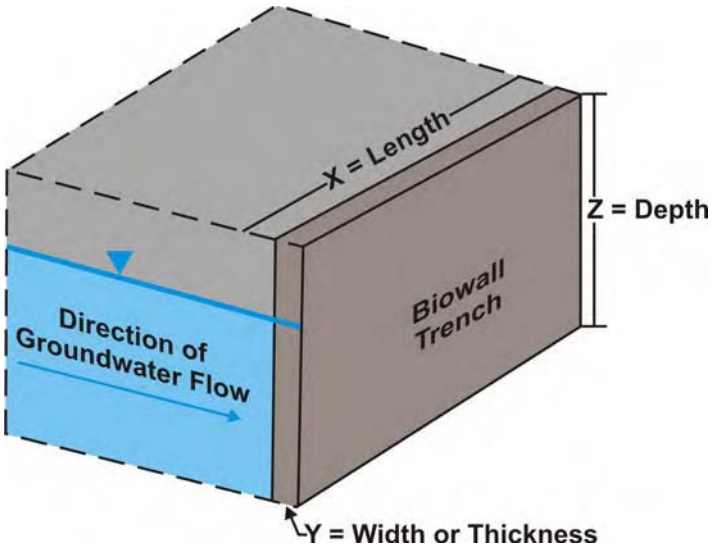

Figure 3.2 Nomenclature for Biowall Dimensions

Note that interception of the entire contaminant plume as defined by an MCL or other regulatory cleanup criterion may not always be practical. Remedial goals may still be met at a downgradient point of compliance, especially when the anaerobic treatment zone extends downgradient of the biowall or treatment of dechlorination products such as *cis*-1,2-DCE, VC or CA in a distal aerobic treatment zone is factored into overall remedial performance. However, the biowall dimensions should intercept the entire contaminant plume to the extent possible.

3.4.1 Biowall Length

To prevent contaminant bypass, the biowall should be long enough to treat the entire width of the plume (dimension perpendicular to groundwater flow) that exceeds performance criteria. If multiple biowall sections are being installed, it is beneficial to overlap the adjoining sections to avoid treatment gaps. For example, **Figure 3.3** shows the configuration of a biowall at Ellsworth AFB, South Dakota. Two separate sections were installed to limit the length of horizontal piping installed for any single section. The biowalls overlap to prevent any bypass, and the biowall at the north end was curved slightly to account for a change in the direction of groundwater flow.

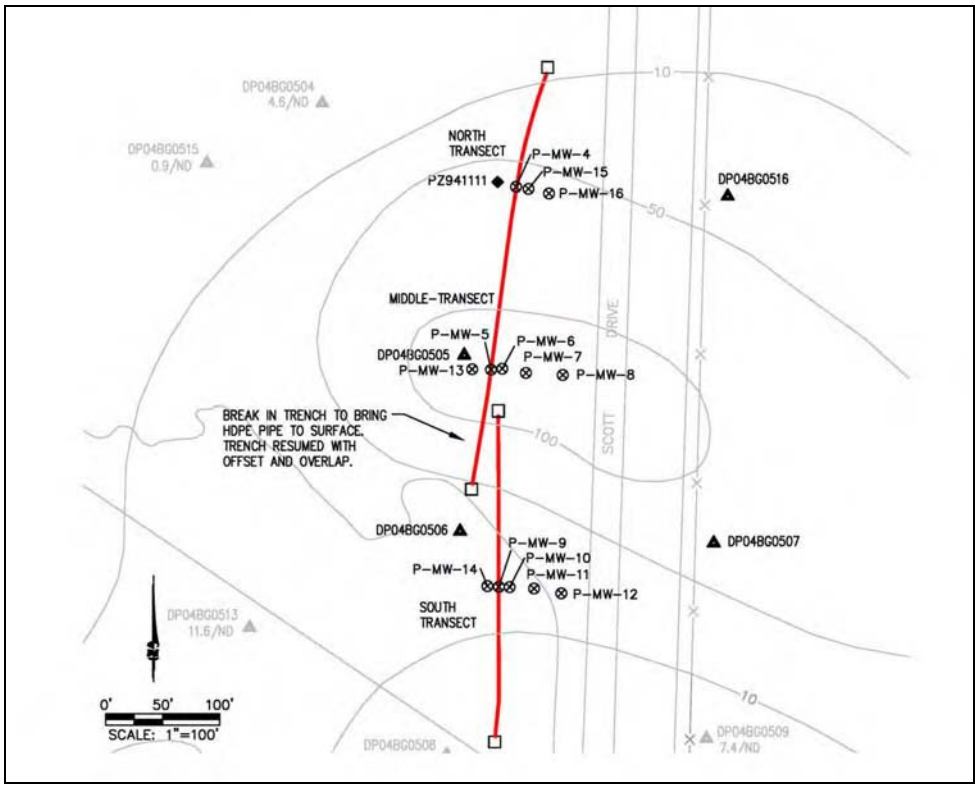

Figure 3.3 As-Built Configuration of Biowall at Ellsworth AFB, South Dakota

3.4.2 Biowall Thickness or Width

The thickness of the biowall must be sufficient to provide the retention time necessary to treat the primary contaminants. The biowall thickness is easily increased when using backhoe excavators. However, continuous one-pass trenchers are typically limited installing trenches 2 to 3 feet thick, and it is more difficult for these trenchers to achieve the maximum depth as the biowall thickness increases. It may be more practical to install a second parallel trench in some circumstances.

3.4.3 Biowall Depth

If possible the biowall trench should be keyed into a competent shale/bedrock layer or similar aquitard. By keying into the competent layer, contaminated groundwater may be prevented from flowing under the biowall. The maximum depth that can be achieved with a continuous trenching machine is about 35 feet. If a lower confining layer is not present, the biowall should extend to the total depth of contamination. Monitoring of groundwater beneath the biowall may be beneficial to document that contaminant bypass has not occurred.

Figure 3.4 illustrates a typical biowall cross section. Design specifications include the depth and thickness of the trench, the location of piping, and the depth to which the mulch mixture will be placed above the seasonal high water table elevation. The primary factor that will affect the trencher's ability to achieve the desired depth is site-specific geology. The subsurface geology should be well documented during monitoring well installation and blow counts collected to estimate formation hardness.

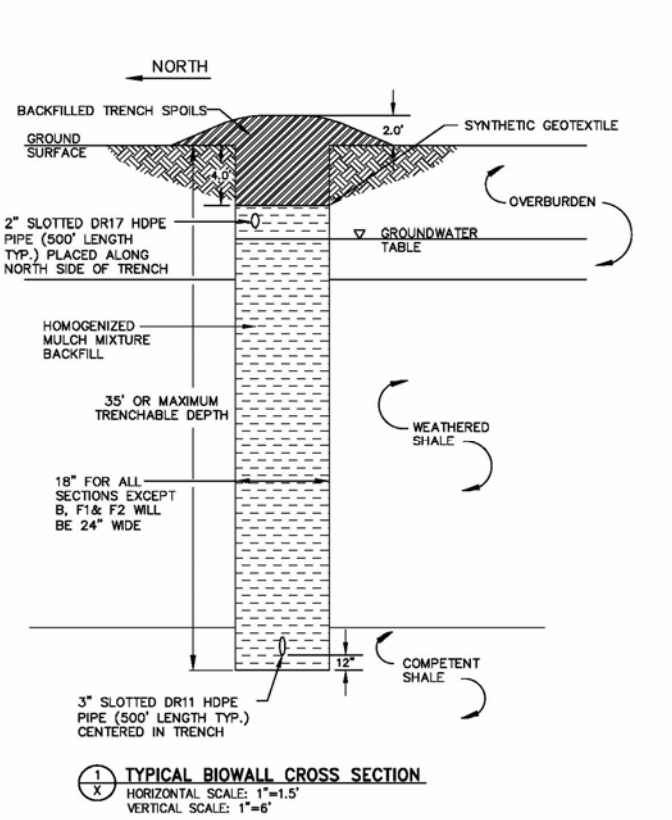

Figure 3.4 Construction Diagram of Typical Biowall Cross Section

Benching is a technique where a portion of the vadose zone soils are removed along the axis of the planned biowall to allow a greater depth of excavation. Continuous trenching rigs are heavy and wide. A bench with a bottom width of 16 feet or more is typically required for a large continuous trencher to operate on. Side-walls of bench excavations usually must be sloped for safety considerations. The cost of permeable mulch biowalls is highly dependent on minimizing the volume of excavated or trenched soils requiring special handling, management, and/or off-

site disposal. Fortunately, benching into uncontaminated soils above a dissolved contaminant plume should not require special handling, and the economics of using benching to achieve the necessary depth may be favorable in some cases.

3.5 DEGRADATION RATES AND RESIDENCE TIME IN THE REACTION ZONE

Effective bioremediation using permeable mulch biowalls or bioreactors depends primarily on sustaining appropriate levels of substrate in the reactive zone, and the development of optimal geochemical and oxidation-reduction conditions for anaerobic degradation processes to occur. But just as important, the reaction zone must be of sufficient size for contaminants to degrade to performance objectives. Insufficient residence time of the contaminants in the reaction zone may result in accumulation of regulated intermediate degradation products, such as *cis*-1,2-DCE or VC.

The primary parameters required to estimate the necessary residence time are the rate at which the contaminant(s) are degraded and the maximum contaminant concentrations. The dimensions of the biowall can then be determined from the rate of groundwater flow through the reaction zone. In practice, the thickness (or width) of a biowall is often a function of the trenching equipment used. For example, most continuous chain trenchers cut a width of 2 feet. A reasonable estimate of the rate of groundwater flow and degradation rate that can be achieved are therefore needed to determine whether one or more biowalls will be adequate to meet performance objectives. The following subsections reference degradation rates listed in the literature, discuss how degradation rates may be calculated from field data, how to estimate the rate of flow through a biowall based on site hydraulic data, and finally how this information is used for design of biowall thickness.

3.5.1 Anaerobic Degradation Rates

Determining a degradation rate that can be achieved within a biowall or bioreactor application is a challenge because each site is unique, with the potential for a wide variation in site-specific conditions. However, results from bench-scale studies and field demonstrations using mulch-based substrates can provide insight into an appropriate degradation rate to use for design purposes.

For example, Shen and Wilson (2007) extracted data from a column study that inferred an overall first-order rate constant for biological reductive dechlorination of TCE on the order of 0.22 to 0.53 per day in the presence of mulch used for the SS-17 biowalls at Altus AFB, Oklahoma. After 383 and 793 days of operation, approximately 50 percent of the removal of TCE was attributed to abiotic reactions with FeS that accumulated in the reactive matrix. Therefore, these rates may not be representative of sites where the potential for production of reduced iron sulfides is low.

Analytical modeling also may be used to estimate site-specific first-order degradation coefficients (k) using known site information (*e.g.*, biowall thickness and specific discharge of the formation) and conservative assumptions regarding material properties (*e.g.*, porosity and dispersivity through the biowall). Ahmad, *et al.* (2007b) describe the use of steady-state analytical model based on the advection-dispersion equation developed by Van Genuchten and Alves (1982). **Table 3.1** summarizes some of the first-order degradation rates from the literature for mulch substrates.

Table 3.1
Literature Values for First-Order Degradation Rates in the Presence of Mulch Substrates

Type of Study	Contaminant	First-Order Rate Coefficient (*k*)	Reference
Column Studies			
Column study using mulch mixture for SS-17 biowall at Altus AFB, Oklahoma	TCE	0.22 to 0.53 per day	Shen and Wilson, 2007
Column study for RDX with 70% tree mulch to 30% pea gravel by volume	RDX	0.20 to 0.27 per hour	Ahmad *et al.*, 2007a
Field Sites			
B301 Pilot Biowall, Offutt AFB, Nebraska	TCE	0.114 per day	Ahmad *et al.*, 2007b using data from GSI, 2001.
B301 Full-Scale Biowall, Offutt AFB, Nebraska	TCE	0.185 per day	Ahmad *et al.*, 2007b using data from GSI, 2004.
OU-1 Biowall, Altus AFB, Oklahoma	TCE	0.230 per day	Ahmad *et al.*, 2007b using data from Henry *et al.*, 2003.

For TCE, it appears that a range of *k* of 0.1 to 0.2 per day is a suitable approximation of the degradation rate that may be achieved in a biowall of approximately 1.5 to 2.0 feet in thickness. However, dechlorination of TCE to DCE to VC to ethene must also be accounted for if sequential biotic anaerobic reductive dechlorination is the primary degradation process. For such target contaminants experiencing reactions-in-series that yield toxic intermediates, the *k* value for each reaction can be estimated by utilizing the BIOCHLOR screening model to model the thickness of the biowall (Ahmad *et al.*, 2007b).

3.5.2 Residence Time

The residence time required to meet remedial objectives can simply be estimated from a reasonable first order rate constant(s) and the maximum contaminant concentration(s) that are present at a site. The solution to the first-order decay rate is:

$$C_t = C_o e^{-(kt)} \qquad (3\text{-}1)$$

where

C_t is the concentration (mass per unit volume or µg/L) at time t (days)

C_o is the initial concentration (µg/L)

k is the first order degradation coefficient (per day)

Equation 3-1 can be rearranged to yield the time (t) to meet a target concentration as:

$$t = -\ln (C_t/C_o) / k \qquad (3\text{-}2)$$

For example, to degrade TCE from 1,000 µg/L (C_o) to 5 µg/L (C_t) at a first order rate of 0.1 per day (k) requires a residence time of approximately 53 days.

The rate of migration of contaminant mass through a biowall trench may be calculated based on site-specific hydrogeology and the properties of the mulch mixture. A simplistic approach may follow the use of Darcy's Law. Darcy's Law states that the volumetric flow rate (Q) through a pipe filled with sand can be calculated as follows:

$$Q = - KA(dh/dl) \quad (3\text{-}3)$$

where

K = proportionality constant (length divided by time [L/T])

A = the cross sectional area of the pipe (L^2)

dh/dl = the horizontal hydraulic gradient (unitless)

More simply stated, Equation 3-3 can be solved to yield the Darcy velocity or specific discharge. As defined, the specific discharge (q) is a volumetric flow rate per unit surface area of porous media:

$$q = Q/A = - K(dh/dl) \quad (3\text{-}4)$$

This equation is useful because the water balance across a biowall of limited thickness can be assumed to be approximately the volumetric flow of water through the aquifer, where values for the proportionality constant are measured as hydraulic conductivity (K). Both K and the horizontal hydraulic gradient (dh/dl) are commonly known from site investigation activities.

Because water only moves through the interconnected pore openings of an aquifer, Darcy's q is a superficial or apparent velocity. That is, q represents the velocity at which water would flow if the aquifer were an open conduit, but does not account for dispersion that causes water to flow through different pore spaces at different rates along individual flow paths that vary in length**.** The velocity of water through the aquifer pore spaces is termed the average linear or seepage velocity where:

$$v = - K(dh/dl) / n_e \quad (3\text{-}5)$$

where

v = pore water (seepage) velocity (L/T)

n_e = effective porosity of the aquifer matrix (unit less)

Typical groundwater seepage velocities for enhanced anaerobic bioremediation applications range from 30 to 1,000 ft/yr. To calculate the seepage velocity across a biowall, one must know or estimate the effective porosity of the mulch mixture. Ahmad *et al*. (2007b) evaluated the effective water-filled porosity of biowall materials in column studies, and reported that an approximation of the effective porosity of biowall backfill material is 40 percent where the mulch fraction ranges from 40 to 60 percent by volume. Shen and Wilson (2007) report a water-filled porosity of 42 percent and an effective porosity of 25 percent for columns constructed with material from the SS-17 biowall at Altus AFB that consisted of 50 percent mulch, 10 percent cotton gin trash, and 40 percent sand

As an example, in **Appendix F.2** the seepage velocity for groundwater across the OU1 biowall site was estimated to be 0.17 foot per day (ft/day) based on a hydraulic gradient of 0.003 foot per foot (ft/ft), an average hydraulic conductivity of 8.7 ft/day, and an effective porosity of 15 percent. Assuming that the specific discharge (q) is the same across the biowall as it is in the aquifer, then the seepage velocity across the biowall can be estimated as 0.10 ft/day using an effective porosity of 25 percent (effective porosity from Shen and Wilson, 2007). Thus, with a biowall width of 1.5 feet, the residence time of groundwater within the OU-1 biowall was estimated to be 15 days. Groundwater residence time may be a conservative estimate of contaminant residence time because it does not account for the effects of sorption and retardation of organic compounds.

These calculations can be taken one step further given that most biowalls are of fixed thickness, and the maximum upgradient contaminant concentration can usually be estimated from site data. The maximum concentration at a given residence time (C_t) can be calculated from Equation 3-1. For the example described above, given a residence time of 15 days and an estimate of 0.20 per day for a first order degradation rate for TCE, then the maximum concentration of TCE that can be treated to an MCL of 5.0 µg/L is approximately 100 µg/L. This represents approximately a 97 percent reduction in the concentration of TCE. Note that substantially higher concentrations of TCE upgradient of the OU-1 biowall (as high as 8,000 µg/L) have been reduced to less than 5 µg/L within the biowall. This suggests that the actual residence time of TCE in the biowall is greater than 15 days due to sorption effects, or that the rate of degradation of TCE is higher than 0.2 per day.

Although these simplistic calculations appear to be conservative, they should always be used with caution because they assume average flow rates. Contaminant breakthrough may occur where flow across the biowall is impacted by preferential flow paths within the aquifer. Where the residence time in a biowall of fixed thickness is not sufficient to degrade maximum contaminant concentrations to performance criteria, multiple biowalls may be required. In this case, the cumulative residence time in two or more biowalls may be used with Equation 3-1. In addition, soluble organic carbon will migrate with groundwater flow downgradient of the biowall trench and establish an anaerobic zone within the aquifer matrix. Additional degradation of contaminants and intermediate degradation products may occur in this zone.

3.6 BIOREACTOR DIMENSIONS

Dimensions of an *in situ* bioreactor (depth, length, and width) also require specification during design, including consideration of the time that contaminants will reside in the treatment zone. However, there may be greater latitude in specifying the dimensions of a bioreactor treatment cell that uses recirculation, relative to passive biowalls that rely on groundwater flow under a natural hydraulic gradient. Three of the four bioreactor applications listed in **Table 1.1** utilize recirculation (Landfill 3 and Building 506 bioreactors at Altus AFB, Oklahoma, and the SWMU bioreactor at Camp Stanley, Texas). In addition, two of the three confidential industrial sites listed in **Table 1.1** use recirculation to pass contaminated groundwater through infiltration trenches. Recirculation captures and treats a greater volume of the aquifer than would pass through the treatment cell by natural groundwater flow alone.

For small source area excavations where the dimensions of the excavation are determined by the extent of soil contamination, the physical dimensions of the bioreactor cell may depend on the final extent soil that is removed. However, for large excavations it may not be practical to line the entire excavation with a mulch mixture. In this case the bioreactor treatment cell may be

installed as a smaller portion of the entire excavation along the upgradient edge of the excavation.

Both the rate of groundwater flow through the bioreactor cell (if below the groundwater table) and the rate of recirculation may be used to establish a water balance through the bioreactor treatment cell. The physical dimensions of the bioreactor cell may be derived from the volumetric flow rate and the residence time required for treatment to performance measures. Given a reasonable estimate of the degradation rate and maximum concentration of the contaminants present, the bioreactor dimensions may be altered as necessary to achieve the desired performance objectives.

3.7 SUBSTRATE MATERIAL OPTIONS

Biowall materials generally include a bulk source of plant material (primarily tree mulch) as a long-term carbon source, materials for enrichment or nutrients (*e.g.*, compost), and coarse sand or pea gravel to maintain permeability and to prevent compaction (**Figure 3.5**). Other agricultural or waste products may be suitable as biowall materials, such as cotton gin trash, mushroom compost, rice hulls, and blended corn cobs. Spraying soybean oil on the mulch mixture is another common method to increase the amount of readily degradable organic carbon, or emulsified vegetable oil may be injected at a later date to rejuvenate the supply of organic substrate. Sand or pea gravel is typically added at a ratio of 40 to 60 percent by volume. Crushed limestone may be considered to buffer pH near neutral. Materials such as gypsum (sulfate) and high-iron sand may be added to stimulate abiotic degradation processes.

Figure 3.5 Materials Used in Construction of the OU-1 Biowall, Altus AFB, Oklahoma

The low solubility of solid substrates requires careful consideration of substrate composition, biowall thickness, and retention time. Solid substrates are intended to be long-term sources of organic carbon, with anticipated life spans exceeding 5 to 10 years. Other investigators have installed trenches and backfilled excavations with sawdust and mulch since the mid-1990s for the treatment of nitrate-contaminated water, and have found little reduction in performance during 7 years of operation (Robertson and Cherry, 1995; Robertson *et al*., 2000).

The amount of organic carbon required to sustain the highly anaerobic conditions required for degradation of chlorinated solvents is greater than that to sustain nitrate reduction alone. Little is known regarding the long-term effectiveness of mulch biowalls and the minimum or threshold concentrations of dissolved organic carbon produced by mulch and compost substrates that are required to sustain anaerobic degradation. Therefore, determining the mulch and compost requirements necessary to sustain anaerobic degradation over periods of 5 to 10 years or more is a critical design and operational objective.

3.7.1 Mulch and Compost

Typically, mulch and compost are mixed with coarse-grained sand or pea gravel at a ratio of 40 to 60 percent by volume. Wood mulch is composed of approximately 40 to 50 percent cellulose (*e.g.*, Duryea *et al.*, 1999), which is a natural polymer of glucose molecules, with the chemical formula $(C_6H_{10}O_5)n$ where *n* ranges from several hundred for wood pulp to over 6,000 for cotton (Senese, 2005). Cotton is the purest natural form of cellulose. In addition to cellulose, wood is primarily composed of hemicellulose (20 to 30 percent), and lignin (25 to 30 percent), with lignin being the component of plant cell material most recalcitrant to biodegradation (Richard, 1996).

The cellulose, hemicellulose, and lignin contents of most North American species of trees have been analyzed and documented in the literature (Petterson, 1984). Variations from published values for a given mulch might represent blends of woods from different types of trees, partial composting of the mulch, or both. The degradation order of biopolymers in mulch generally follows in order of hemicellulose greater than amorphous cellulose, greater than crystalline cellulose, greater than lignin (Winandy and Lebow 2001). Therefore, a qualitative analysis of the relative bioavailability of organic carbon in a mulch source may be made if the origin (species) of the mulch is known (GSI, 2005; Ahmad *et al*., 2007a).

Heartwood consists of the central core of the tree that has been hardened using insoluble resins by the tree because this section contains dead cells from past cell embolisms. Conversely, sapwood grows around the heartwood (tree-ring formation) and contains living cells that are porous, and will break down more readily in a subsurface setting. Therefore, the leaves and soft tissue of the mulch are more amenable to biodegradation, and the mulch should contain a high percentage of fresh "green" or "soft" material. Alternatively, partial composting of the mulch will break down the plant cell walls and produce more readily degradable material.

Composted plant material, or alternative organic amendments, should be added to mulch that contains a high percentage of dry woody heartwood material. Compost provides a source of readily degradable organic carbon in the form of cellulose to rapidly stimulate anaerobic conditions. Compost has little or no hemicellulose (*i.e.*, xylans), the cross-linking molecule that binds cellulose microfibrils to each other and to the inert lignin content of mulch. The absence of hemicellulose allows the remaining cellulose in compost to be readily available for hydrolysis. Compost also supplies the inoculum for increased bioactivity for mulch hydrolysis and biodegradation.

As an example, cotton gin trash was added to mulch for the SS-17 biowall installation at Altus AFB, Oklahoma. Shen and Wilson (2007, supporting data) analyzed the mulch and cotton gin trash for total carbon, total nitrogen, total ash, and fiber content (**Table 3.2**). The fiber concentrations of cellulose and hemicellulose were determined by differences between Acid Detergent Fiber (ADF) and Acid Digestible Lignin (ADL), and between Neutral Detergent Fiber (NDF) and ADF, respectively. Note that the tree mulch had a relatively low percentage of lignin compared to the typical percentages described above. This may be due to degradation (composting) of the material that occurred over several months from the time the material was staged until it was collected and sampled for the column studies. Fiber analyses used for evaluation of animal forage may be used to evaluate the fiber content of differing sources of mulch and compost (**Section 6.5**).

Table 3.2
Components of Tree Mulch and Cotton Gin Trash used at Altus AFB, Oklahoma
(from Shen and Wilson, 2007 – supporting data)

Component	Percentage (%) in Plant Biomass on a Dry Weight Basis (Mean ± Standard deviation, n=3)	
	Tree Mulch	Cotton Gin Trash
Cellulose	37.1 ± 2.05	39.6 ± 1.63
Hemicellulose	19.4 ± 0.85	19.9 ± 2.71
Lignin	4.7 ± 1.93	9.6 ± 0.23
Total Ash	28.48 ± 1.88	14.2 ± 0.25
Total Nitrogen	0.44 ± 0.02	1.3 ± 0.02
Total Carbon	34.9 ± 2.08	41.1 ± 0.50

3.7.2 Alternative Organic Amendments

Tree mulch is relatively recalcitrant to biodegradation and may not contain sufficient bioavailable carbon to sustain the reaction zone. Partial composting of the tree mulch will break down lignin bonds and provide more bioavailable organic carbon. However, alternative biowall materials with higher amounts of bioavailable organic carbon may need to be added to the mulch mixture.

Because tree mulch alone may not provide an adequate carbon source, alternate carbon sources should be added to the biowall mixture. These materials include compost, vegetable oil, or agricultural waste materials such as cotton gin trash, mushroom compost, or poultry litter.

The selection of appropriate amendments should be based on a search of local agricultural products and common carbon-based waste streams. Not all potential amendments will be locally available or be the most cost-effective option in different areas of the country. Two examples that have been used for Air Force and Army biowall applications are described below (cotton gin trash and vegetable oil). Examples of other potentially available alternative amendments include composted leaf and grass clippings, mushroom compost, hay or other silage, feed corn, off-specification grains, poultry litter, stable bedding materials, composted manure, spent grain from breweries, or bulk chitin from seafood processing.

Waste material from local cotton gin processing is often available and is referred to as "cotton gin trash." It is composed of materials remaining from the cotton bolls after the lint is removed, along with stems and leaves. Cotton gin trash is typically composted for application as an agricultural amendment and fertilizer. Peak production of cotton gin trash is in November and December, coinciding with the stripper cotton harvesting. By adding the cotton gin trash to the mulch mixture, a readily degradable source of organic carbon and nitrogen are added to the subsurface (**Table 3.2**).

Stripper cotton is not collected until after the first freeze, and may have been sprayed by defoliants to allow earlier harvesting. Defoliants that may have been used on the cotton prior to harvesting are included in **Table C.2** in **Appendix C**. These products have rapid degradation rates and are readily decomposed during composting. Defoliants should be fully degraded by the

time the material is stockpiled and ready for biowall installation. Cotton gin trash is routinely fed to livestock, another indication that the toxicity of the defoliants is only temporal.

The use of vegetable (edible) oil for *in situ* bioremediation is described in the *Protocol for In Situ Bioremediation of Chlorinated Solvents Using Edible Oil* (AFCEE, 2007). Edible oils are a long-lasting carbon source due to low solubility in water, and should typically last 2 years or more. Several biowall applications to date (*e.g.*, **Appendix F.1**) have coated the wood mulch with soybean oil prior to installation.

3.7.3 Sand, Gravel, and Limestone

Sand and gravel are added to the backfill for biowalls and bioreactors to provide a weighting material for emplacement, to reduce the amount of compaction after installation, and to enhance and maintain the permeability of the mixture. Sand and gravel are typically added at 40 to 60 percent by volume of the substrate mixture, with the sand fraction filling voids between the mulch particles and providing matrix support. This maintains a high permeability for groundwater migration or infiltration through the mixture, as well as stabilizing the material and preventing compaction. It is important to maintain a higher permeability in the trench relative to the surrounding formation to limit contaminant bypass. Sand provided for inclusion in the mulch mixture should contain no more then 5 percent fines (capable of passing a 200 sieve) to prevent clogging of pore spaces, and may contain gravel up to 1-inch in diameter.

Limestone gravel may also be used to as a weighting material and has the added benefit of providing calcium carbonate as a buffering agent for stabilizing pH. A lowering of pH may occur due to formation of metabolic acids, which may inhibit degradation processes in some cases. Calcium carbonate from the limestone is slowly dissolved by acids generated by degradation of the organic substrate; hence the limestone provides a long-term buffering agent.

3.7.4 Inorganic Amendments

Inorganic amendments to the mulch mixture may be added to stimulate biogeochemical transformation (abiotic) processes, including sulfate and iron. Both of these are found naturally in aquifer systems, but concentrations are highly variable. Sulfate in groundwater at concentrations above 500 to 1,000 mg/L may be sufficient for biogeochemical transformation. If additional sulfate is required, it may be added to the biowall in the form of crushed gypsum or gypsum pellets commonly used as agricultural amendments. Pellets are preferred as the slower and longer the sulfate dissolves the greater the potential for reactive iron sulfides to accumulate.

Ferric iron has two purposes; it is reduced and precipitates with sulfide to form FeS as an abiotic reactant, but it also reduces the concentration of hydrogen sulfide and prevents toxicity to the biotic reductive dechlorination process (*e.g.*, Maillacheruvu and Parkin, 1996). A source for ferric iron may be found in the biowall weighting material (*e.g.*, river sand), and in sediments immediately downgradient of the biowall. The easiest and most cost effective way to increase the amount of available iron is to choose a biowall sand material that is naturally high in iron. Attempts have been made to increase the mass of ferric iron in the biowall material by blending iron ore with the backfill material. For example, magnetite ore was mixed into the biowall mixture at Altus AFB, Oklahoma and at Ellsworth AFB, South Dakota on an experimental basis. The effectiveness of adding iron ore is yet to be determined, and may have more to do with the surface area and bioavailability of ferric iron than the bulk mass of iron alone.

Methods for determining how much iron or sulfate should be added to stimulate biogeochemical transformation of CAHs has been not been proven, although attempts to quantify the process for biowall design have been attempted at Dover AFB, Delaware (Parsons, 2007a) and at Ellsworth AFB, South Dakota (Parsons, 2006c). The stoichiometry and mass calculations that may be used to evaluate the potential for FeS to form, and simplistic calculations to evaluate whether a sulfate or iron amendment should be added, are described in **Appendix D**. These calculations should be used with caution, as research into biogeochemical transformation of CAHs and demonstrations to stimulate these processes are only in the early stages of development (AFCEE *et al.*, 2008).

3.8 INSTALLATION METHODS

The general approach used for construction of permeable mulch biowalls and bioreactors is to use established construction techniques to place the bulk materials in a trench, excavation, or surface amendment. Conventional trenching techniques and continuous chain trenchers have been used to construct biowalls and bioreactors. Large backhoes equipped with long-arm booms are also capable of trenching to depths of 40 feet (12 meters) or more, but the use of a bioslurry is generally required to maintain an open trench. This method is typically not cost competitive relative to other trenching methods or the construction of biobarriers using fluid substrates injected directly into the subsurface.

The depth to which a trench can be constructed in the saturated zone is dependent on lithologic conditions. Unconsolidated but cohesive sediments or weathered bedrock are generally suitable. Highly compacted or cemented lithologies, or non-cohesive sediments, will limit the ability to trench or excavate to required depths.

Deeper applications are possible by benching down prior to deploying the trenching equipment. The depth to which a bench can be excavated is limited by the difference between the ground surface elevation and the water table elevation. Several feet of unsaturated soil must remain above the water table to provide a stable platform for trenching equipment. Trenching may interfere with site infrastructure and utilities, and trenching equipment typically requires a 20-foot (6-meter) wide footprint on stable ground. Therefore, biowalls may not be readily installed at some sites.

Trenching methods should be carefully selected and implemented to avoid potential lowering of the permeability of the trench wall. The development of surface ‘skins’ that lower the relative permeability of the trench wall may result from infiltration of bioslurries that produces a filter cake on the trench wall, or by smearing of silts and clays across layers of higher permeability (*e.g.*, sands) by the trencher cutting tools.

3.8.1 Conventional Construction Techniques

The easiest and most cost effective construction technique is the use of a conventional backhoe in soils that do not cave or slough. In general, the greater the saturated thickness to be trenched or excavated, and the sandier and less consolidated the sediments, the less depth can be achieved without the use of shoring. For small trenches less than a few hundred feet in length and less than approximately 20 feet deep, conventional trenching with trench boxes and shoring may be the most economical alternative. Equipment is typically available locally, and these methods may be less expensive relative to the high cost of mobilizing specialized chain trenchers and long-arm excavators. Biowalls have been installed in this manner at Seneca Army Depot

Activity, New York, at Whiteman AFB, Missouri, and at Naval Joint Reserve Base Fort Worth, Texas. But in general, the number of sites where conventional trenching can be used will be few.

Caution is advised when emplacing a sand and mulch mixture when a standing column of water fills a conventionally excavated trench or pit. Due to the difference in density of the two materials, there is potential for the sand and mulch to separate, resulting in non-uniform distribution of the mixture. Several methods may be employed to reduce the potential for separation of the materials. These include 1) shredding the mulch to a finer size and pre-hydrating the mulch to increase its density, 2) lowering the mulch mixture in a large backhoe bucket to the base of the excavation before emptying the bucket, and 3) starting at one end of the excavation, bring the mulch to above the water table and then use a backhoe bucket to "push" the bulk mulch mixture down the face of the backfill as the mulch mixture is added.

3.8.2 Continuous Chain Trenching

Continuous, one-pass trenching machines used to lay utility lines or for installing dewatering trenches are a rapid and effective way to install a biowall trench. Chain trenchers are capable of installing biowalls in a one-pass operation where the trench is cut and the biowall material emplaced in one continuous operation (**Figure 3.6**). Continuous chain trenchers are currently capable of trenching to depths of 35 feet (11 meters), or up to 40 to 45 feet (12 to 14 meters) with benching, and are being modified with special cutting teeth to trench in soft or weathered bedrock materials. The cutting boom excavates a trench by simultaneously rotating the cutting chain and advancing a steel box and hopper assembly. This provides for stabilization of the trench sidewalls during excavation and placement of the sand and mulch mixture, which is introduced through the feed hopper. Standard widths of trenches/biowalls that can be installed using this technique are typically 1.5 feet, 2.0 feet, or 3.0 feet. A width of 2.0 feet is often optimal due to higher mobilization costs for larger equipment and slower advancement rates associated with trenching to a width of 3.0 feet.

3.9 ALTERNATIVE SUBSTRATE DELIVERY OPTIONS

Fluid substrates (*e.g.*, emulsified vegetable oil) may be added to supplement or replenish a biowall or bioreactor system. This may be incorporated into the design by installing dedicated piping or injection wells. The highly permeable coarse sand/mulch mixture relative to native materials promotes a uniform distribution of the injected fluids within the biowall trench.

One design alternative is to install perforated HDPE pipe at the bottom of the mulch mixture, and/or install a slotted HDPE or polyvinyl chloride (PVC) pipe at the top of the mulch mixture. HDPE pipes are typically 2- to 3-inches in diameter, factory slotted to design specification, and laid in lengths of approximately 200 to 500 feet. Continuous one-pass trenchers are designed to install this pipe for de-watering purposes. Installation of the bottom piping does not hinder trench excavation (see **Figure 3.6**) and additional cost to install the pipe is usually less than $10 per linear foot. The pipe ends are brought to the ground surface to be accessible from both ends of the biowall.

Figure 3.6 Continuous Chain Trencher at Ellsworth AFB, South Dakota

Another design is to install permanent injection wells into the biowall or bioreactor (**Figure 3.7**). Because the substrate will tend to flow along the more permeable material in the biowall trench, injection wells may be spaced at distances of 20 to 30 feet on center along the length of the biowall. Where the trench excavation stays open after excavation with conventional equipment, a combination of vertical risers and horizontal piping may be installed prior to backfilling the biowall material. Subsurface conditions at NWIRP McGregor allow perforated piping to be installed in this manner.

Where the biowall trench does not uniformly extend to the total depth of contamination, injection wells may be installed beneath the biowall to extend the treatment zone to a greater depth. Either vertical or horizontal wells could be considered for this application. However, biobarriers constructed by direct injection of slow release substrates are likely to be more economical where large portions of the contaminant plume migrate below the limits of trenching. Similar consideration of the depth of contamination should be applied for construction of bioreactor cells.

Finally, the biowall materials are readily penetrated by direct-push techniques. Fluid substrates may be injected into the biowall through direct-push rods and screened or slotted drive points. When substrate replenishment is anticipated to be infrequent, this may be a cost effective alternative to installing permanent injection wells or piping.

3.10 REGULATORY CONSIDERATIONS

Trenching and excavation operations can produce several hundred to thousands of cubic yards of excavated soil that may potentially contain hazardous levels of contaminants. In most cases the excavated trench soil will not constitute a hazard. However, a residuals management plan (**Section 5**) should be developed for confirmation sampling. Soil lay down or staging areas should be incorporated into the design, with contingencies for segregating and handling any

hazardous soil that is encountered. If trenching spoils must be transported off site for disposal it may render the biowall technology cost prohibitive relative to other *in situ* bioremediation techniques using direct injection. On-site treatment of soil such as land farms or treatment cells amended with mulch or other substrates may be considered in some cases.

3.11 BIOWALL AND BIOREACTOR MONITORING CONFIGURATIONS

System design of a biowall or bioreactor includes development of a monitoring network to document performance and attainment of remedial and performance objectives. System monitoring is conducted to establish baseline conditions for comparison to performance monitoring results. Initial monitoring over a period of one to two years may be more frequent (perhaps quarterly) and more extensive than longer term monitoring to determine the optimal conditions for anaerobic degradation (**Section 6.2**). An optimized monitoring protocol and less frequent monitoring (perhaps bi-annual to annual) may be used for long-term O&M monitoring to determine if, and when, substrate replenishment may be required (**Section 8**). The configuration of the monitoring network should be adequate to document performance objectives and to meet long-term O&M requirements.

Figure 3.7 Installation of Vertical Injection Risers and Horizontal Pipe at NWIRP McGregor, Texas (photo courtesy of US Navy)

3.11.1 Monitoring Network Design

Monitoring locations for baseline characterization and performance monitoring should be located upgradient, within, and downgradient of the biowall or bioreactor reaction zone, parallel to the direction of groundwater flow. These wells are intended to monitor changing groundwater chemistry over time along the path of groundwater flow through the biowall or bioreactor treatment area. **Figure 3.8** illustrates an example monitoring network for a biowall; similar considerations also apply to bioreactor cells. Consideration should be given to the groundwater seepage velocity and the desired frequency of performance monitoring when determining monitoring locations and spacing. Closer well spacing and/or less

frequent monitoring may be warranted for sites with low groundwater velocities relative to sites with high groundwater velocities. Rationale for well placement and examples of effective monitoring networks are described in AFCEE (2000) and Wiedemeier and Haas (2003).

It is useful to have monitoring location within the biowall trench or bioreactor cell as well. Because of the differences between the material installed in the excavation and the surrounding natural formation, it is possible that differing degradation processes occur within the two media. Samples collected from within the biowall or bioreactor excavation will be most representative of the processes that are occurring there. Note that substantial changes in groundwater chemistry may occur from the upgradient to downgradient edges of the reaction zone. Samples collected from within a biowall are likely to represent a combination or average of the processes that are occurring from the upgradient to downgradient edge of the biowall trench.

It is also common to see higher reductions in contaminant concentrations within the biowall or bioreactor media relative to downgradient locations. This is likely due to desorption of contaminant mass from the native formation and back-diffusion of contaminants from low permeability sediments downgradient of the biowall trench or bioreactor cell. While concentrations in downgradient locations should be used to determine the overall impact of the remedy on groundwater quality, samples from downgradient locations may not accurately reflect the degradation processes that are occurring within actual biowall trench or bioreactor cell.

Monitoring well screened intervals should be adequate to monitor the saturated interval treated by the biowall or bioreactor. Wells screened in distinct vertical horizons may be required to monitor groundwater flow and contaminant migration along preferential pathways (**Figure 3.8**). Vertical plume migration in the presence of vertical hydraulic gradients where a lower confining layer is not present may also require wells at deeper depths. For saturated zones greater than 15 to 20 feet it is beneficial to have wells screened at multiple depths to determine vertical hydraulic gradients, the potential for vertical migration of the plume, and to monitor for potential contaminant bypass at the base of the biowall trench or bioreactor cell.

Monitoring well installation within or directly adjacent to a biowall or bioreactor must be completed after the biowall trench or bioreactor cell are installed due to the heavy equipment used during construction. While a thorough understanding of baseline conditions is an important consideration, excavation and construction may destroy existing wells within 10 to 15 feet of the biowall or bioreactor. Special attention should be applied to prevent the destruction of monitoring wells throughout the site due to intensive traffic and operation of heavy equipment.

Multiple transects or at least some cross-gradient well locations are useful to define the lateral extent of treatment. Cross-gradient wells may be used to document that contaminant bypass around the ends of the biowall is not occurring. Downgradient locations within the treatment zone are sampled to determine the area of effective substrate mixing and biostimulation. Monitoring locations for long-term O&M monitoring may be limited to a subset of the existing monitoring network.

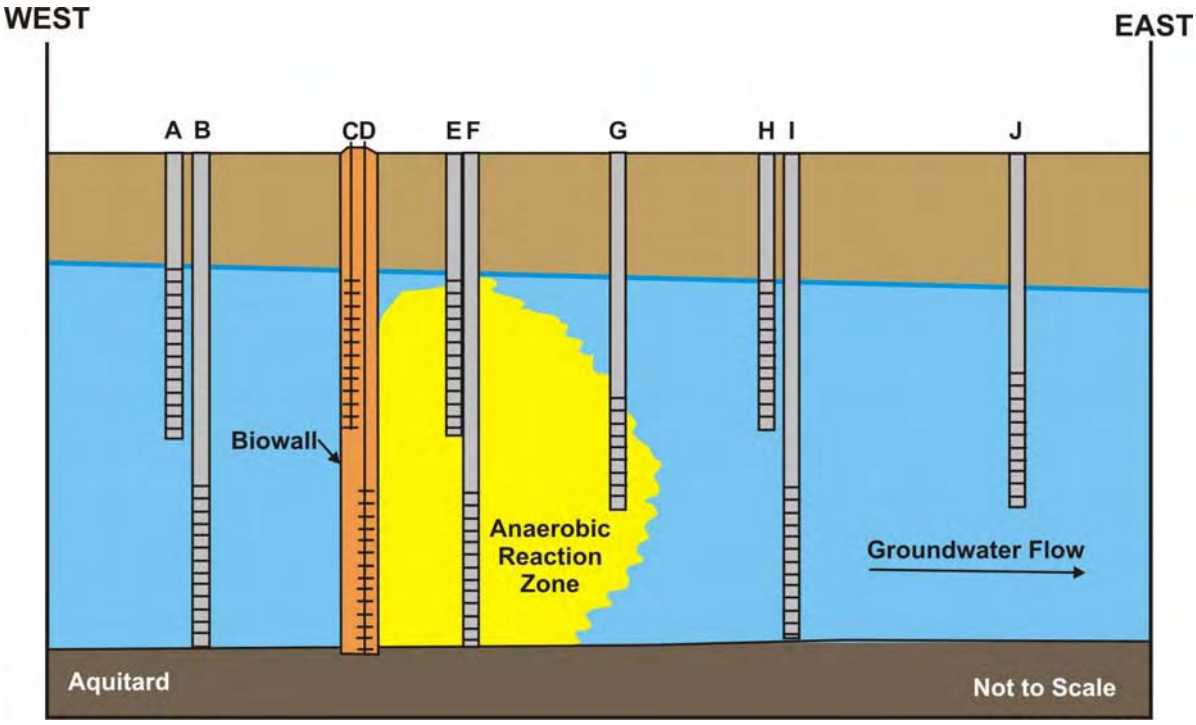

Figure 3.8 Cross-Section View of an Example Monitoring Well Transect for a Permeable Mulch Biowall

3.11.2 Monitoring Frequency

Because biowalls and bioreactors are typically passive or low-maintenance remedies, performance monitoring is typically conducted on a quarterly to annual basis for most systems. For permeable mulch biowall and bioreactor systems where there is no operational component during the first few years, quarterly to semi-annual monitoring is sufficient to begin with. Microbial growth and acclimation within the biowall or bioreactor may take 6 to 12 months or more for the system to achieve optimal performance. Frequent sampling at periods of less than a few months may yield unsatisfactory early results and result in an unjustified lack of confidence in the effectiveness of the system.

Long-term sampling protocols (**Section 6.2**) and monitoring frequency should be optimized based on the more extensive and more frequent monitoring that is initially performed. In some instances, longer-term performance monitoring of passive systems may be tied to annual base-wide monitoring programs.

SECTION 4

CONSTRUCTION MANAGEMENT PLAN

4.1 SITE MANAGEMENT FOR BIOWALL OR BIOREACTOR INSTALLATION

The objective of a construction management plan is to ensure that proper materials, construction techniques, methods, and procedures are implemented by the contractor and completed in accordance with project design specifications. This plan also provides a means to identify problems that may occur during construction and provides appropriate methods for resolution of these problems. Many of the sampling and analysis procedures for quality assurance and quality control (QA/QC) implemented during construction of a biowall or bioreactor are described in an FSP (**Section 6**).

Construction of biowall and *in situ* bioreactor systems requires a competent construction manager to oversee the installation, including appropriate training in construction using open trenching and excavations. Other team members should include competent engineers for design and development of engineering specifications, geologists for evaluation of subsurface geology and groundwater hydraulics, and a health and safety supervisor to ensure safe working practices.

4.2 SITE PREPARATION

An example of site preparation activities for a biowall system are listed in **Table 4.1**; similar considerations would apply to bioreactor construction. Many activities include visual observations to insure that all site preparation activities are completed prior to beginning installation. Site preparation includes finalizing the alignment of the biowall trench or bioreactor cell, final utility locates, clearing the excavation alignment, and stockpiling material at the site. Other site-specific activities may be required depending on site infrastructure and use.

4.2.1 Infrastructure and Utility Clearances

A safe distance should be kept from any buildings or structures to maintain the integrity of foundations and footings, as well as to reduce the potential for migration of toxic or noxious vapors from the anaerobic treatment zone to potential exposure points. Digging permits and utility locates for all intrusive construction or drilling activities must be obtained prior to installation of a biowall/bioreactor and all associated groundwater monitoring wells. This includes overhead as well as underground utilities. Utility locates are typically provided by base utility engineers or off-base public/private utility companies. Locations of communication lines at many DoD facilities are often not recorded or maintained by off-base public utility or locating companies. Therefore, clearance for communication lines should be addressed by the appropriate facility personnel.

Table 4.1
Examples of Preparatory Inspection Activities for Biowall or Bioreactor Construction

Preparatory Inspection Activity	Method	Frequency	Acceptance Criteria
Utility Survey	Facility Utility Engineers or Public Utility Companies	Prior to finalizing biowall alignment.	Confirm the location of all utilities.
Demarcation of Biowall	Site Survey – Survey in alignment and mark with grade stakes	Once along biowall or bioreactor alignment after review of final design.	Establish grade stakes along the designed biowall or bioreactor alignment according to the design drawings. For biowalls, grade stakes should be placed at the start and termination of each segment, at 50-foot intervals along each segment, at 20-foot intervals along curves, and at any change in biowall direction.
Biowall Alignment Clearing and Grading	Visual	Once along biowall alignment prior to construction of the section.	Confirm that the alignment of the biowall or bioreactor has been cleared of obstructions and is level to 20 feet on either side of the excavation alignment. The cleared pathway or area should be flat with a consistent grade.
Disposal Area Pre-Acceptance Approval	Visual – Obtain facility approval	Once prior to trenching.	Confirm approval and location for disposal of excavation spoils. Confirmation should include guidelines for analytical results that will be acceptable and manner of soil placement.
Mulch Stockpile and Homogenization Area Approval	Visual – Obtain facility approval	Once prior to commencing stockpiling.	Confirm approval and location for stockpiling mulch, sand and other backfill materials (*e.g.*, cotton gin trash, vegetable oil). Confirm approval for equipment storage during construction. Confirm that material haul routes are approved by the facility security police.
Site Access Approval	Facility Access Passes	Prior to mobilization, staging, and installation.	Confirm approval for facility access for field team and subcontractors.
Job Site Trailer Approval	Visual – Obtain facility approval	Once prior to trenching or excavation.	Confirm approval and location for site trailer and availability of electrical power.

Most utilities will be located above the groundwater surface. In some cases, utilities may be temporarily moved or breached to allow installation of the biowall or bioreactor below the utility. Shallow storm and sanitary sewers may often be temporarily breached and replaced at relatively low cost compared to the overall biowall/bioreactor installation cost. In the event that a utility cannot be breached in a cost effective manner (*e.g.*, fiber optic communications or gas lines), the space in the biowall or bioreactor alignment may be replaced by injection of a fluid substrate below the utility (*e.g.*, emulsified vegetable oil) or blocked by pressure injection of a grout material below the water table and the utility lines.

4.2.3 Staging Areas

Prior to installation of a biowall or bioreactor, fill materials must be procured and staged at the site. An adequate site near the system installation should be identified for staging and mixing of the materials. The area should be readily accessible to heavy construction equipment, with flat topography and adequate drainage. Typically the materials are staged in parallel windrows adjacent to and along the biowall alignment or bioreactor cell. This facilitates mixing and transfer to the excavation during construction.

4.2.3 Transportation Plan

A transportation plan should be prepared that covers the transport of the mulch mixture to stockpile locations at the staging areas, and transportation of excavated soil if required. This includes the use of public and facility roadways, and right-of-ways granted for construction of the biowall systems. Tracking of materials such as contaminated soil onto local haul roads or public highways should be avoided. Vehicles may need to be inspected and gross decontamination (*e.g.*, brushing soil off tires with a broom) may need to be performed. Generation of dust along the haul roads should be monitored by visual observation. Dust control measures such as moisture addition with a water truck may be needed.

4.2.4 Health and Safety

Site security is necessary to prevent exposure of unauthorized, unprotected individuals to the work area. Site security should be enforced by the site health and safety officer or a designated alternate to ensure that only authorized personnel are allowed in the work area. This person should also ensure that entry personnel have the required level of personal protective equipment (PPE), and are trained under the requirements of 29 Code of Federal Regulations (CFR) 1910.120. All visitors to the work site on base should be required to report to the project manager and/or the site superintendent as soon as they arrive on site.

Health and safety is paramount to installation of biowall and bioreactor systems. The work area should be clearly marked through the use of signs, barrier rope, tape, or fencing. Trenches or excavations should be secured by designated safety personnel while workers are onsite. These personnel should be responsible for monitoring the length of the open excavation during installation. Site personnel and sampling technicians should maintain a safe distance of at least 20 feet from the excavation and construction equipment when in operation.

Use of continuous trenching and backfill methods can minimize open trench conditions during installation of a biowall. At the end of each working day it is advisable to fill the trench

or excavation to the extent possible to avoid leaving an open excavation during off duty hours. Fencing should be installed around the excavation area when workers are off-site for the evening and/or on weekends.

4.2.5 Surveying Excavation and Monitoring Well Locations

The location and extent of the biowall trench or bioreactor cell should be surveyed by a registered surveyor prior to installation. Typically, stations designated on design drawings are located by a surveyor and marked with wooden stakes, flagged, and labeled. Each biowall segment should have a station located at the beginning of the segment, at the end of the segment, and typically at 50-foot intervals in between for the trenching subcontractor to follow during installation (**Table 4.1**). In addition, station coordinates should be provided for points where the biowall may change direction. Similar stations may be marked for the corners or outline of a bioreactor cell.

The initial survey should be followed by a utility locate conducted by appropriate facility engineering personnel or public/private utility companies. Utility drawings should be collected for use during on-site clearances, and a copy of the utility drawings should be maintained on site during installation of the biowall and associated groundwater monitoring points. Based on the final utility locate and biowall survey, the location of the biowall or bioreactor may be need to be adjusted during construction to accommodate utilities or other obstacles.

Biowall locations or edges of a bioreactor excavation should be marked during construction, typically using metal posts painted a high visibility color. After construction the biowall or bioreactor locations should be re-surveyed to complete as-built design drawings, including the location of any monitoring wells that are installed. Once site restoration is completed, it is usually not possible to determine where the biowall or bioreactor is located based on visual observations. Typically the site is re-seeded with native grasses, landscaped, or paved according to how the site is used. The survey data may be necessary if the biowall or bioreactor must be precisely located at a future date.

4.3 BIOWALL OR BIOREACTOR INSTALLATION

4.3.1 Homogenization of Mulch Mixture

The mulch mixture is typically prepared at a central staging area(s). A common mulch mixture is approximately 50 percent by volume wood mulch and 50 percent by volume sand or gravel (see **Section 3.7**). The delivery and mixing of the mulch should begin prior to the commencement of excavation and construction. A sufficient volume of the mulch mixture should be staged at all times to keep up with backfill operations to avoid construction delays.

Prior to mixing and homogenization of the mulch mixture, the mulch should be maintained in an actively composting state by keeping it moist (but not saturated). This provides several important benefits that includes 1) aerobic decomposition of the mulch to provide a more readily biodegradable source of organic carbon, 2) a qualitative confirmation that nutrient content is sufficient for high microbial activity, and 3) maintaining a higher moisture content and higher density mulch. A dry, friable mulch offers distinct disadvantages in that it is less dense and more buoyant. A dry mulch may result in extensive separation of sand and mulch whenever

groundwater is encountered during emplacement. Maintaining a high moisture content also reduces the potential for a wind-blown mulch hazard, although the mulch stockpiles may still need to be covered in windy areas.

One homogenization method is to start with separate piles of sand and mulch with an empty area in the middle large enough to contain the sum of the two piles. A pay loader is used to take one bucket of sand from the sand pile and dump it in the middle. The operator should reverse while dumping so that the bucket of sand forms a layer about 6 to 12 inches thick. The pay loader than gets a bucket of mulch and dumps it on top of the bucket of sand in the same way. The process should be repeated and at the same time an excavator is used to turn the pile. This method appears time consuming, but experience has shown that it proceeds rapidly and does a reasonable job of mixing the material.

Other mixing methods may be used. For example, biowall materials may be mixed in roll-off boxes using a small front end loader (Bobcat) to add and mix the materials. This method contains the materials and limits disturbance of the site. This may be desirable in developed areas such as parking lots or parks.

If additional amendments are to be added, it may be easiest to add them directly to windrows or stockpiles of either the sand or mulch material. **Figure 4.1** shows windrows of mulch, sand, gravel (for weighting) and iron ore at the BG-05 Site, Ellsworth AFB, South Dakota. Low volume fractions of iron ore or gypsum pellets added to stimulate abiotic processes, or limestone gravel to buffer pH, are best mixed with the sand fraction prior to mixing with the mulch. Their physical properties are closer to sand than mulch, and they will likely mix more uniformly throughout the sand fraction. For applications where soybean oil has been added to the mulch mixture (*e.g.*, the Ash Landfill at Seneca Army Depot Activity, New York - **Appendix F.3**), the oil has been sprayed onto either the mulch piles or the sand/mulch mixture, as the oil sticks to either material. Spraying the oil in several passes or lifts, and turning of the piles several times may be required to get uniform distribution of the oil.

Field personnel should determine when the mixture is adequately homogenized and then collect grab samples for visual inspection and QC testing based on bulk density or weight ratio of sand to organics. An example of inspection of the mulch mixture is described in **Section 6.2**. QC samples are typically collected from mulch stockpiles placed along the biowall at one sample per 50 to 100 feet of biowall.

4.3.2 Trench Installation

Biowalls are typically installed by either a conventional backhoe or continuous one-pass trencher (**Figure 4.2**), depending on biowall design specifications and site conditions. The minimum biowall thickness and minimum depth should be specified. Sufficient clearance should be allowed for the trenching machinery, the equipment used to load the biowall mixture, and for staging of the mulch mixture and the trench spoil. A clearance of 20 to 25 feet on either side of the biowall trench is typically required.

Figure 4.1 Stockpiling of Biowall Materials Prior to Mixing at the BG-05 Site, Ellsworth AFB, South Dakota (from left to right is mulch, sand, gravel, and magnetite ore)

Figure 4.2 Slotted HDPE Pipe Threaded Through a Continuous One-Pass Trencher Prior to Installation, Ellsworth AFB, South Dakota

If the biowall is to be keyed into bedrock or a low permeability formation, additional quality control steps should be taken to ensure that the appropriate depth is achieved. One method used for a biowall at the Pueblo Chemical Depot, Colorado, was to create an interpolated bedrock trace using lithologic data from pilot borings completed along the biowall alignment prior to trenching. Coring at the base of the biowall trench, perhaps during monitoring well installation, may also be used to document that the biowall has been installed into the low permeability horizon.

The mulch mixture is continuously backfilled to prevent caving in an open trench or excavation. For continuous one-pass trenchers, the mulch mixture is fed via a hopper on the back or side of the trencher, and is added simultaneously with the HDPE distribution piping on the bottom of the biowall (if installed). The mulch mixture is placed from the bottom of the biowall to above the highest anticipated water level, or to within 3 to 4 feet bgs for safety reasons.

If slotted HDPE distribution pipe is installed at the bottom of the biowall trench, tension should be used to keep the distribution piping centered in the biowall. Piping is typically placed in sections not to exceed 400 to 500 feet in length, with both ends of the pipe extending up to the surface so that fluids can be pressure injected from either end. **Figure 4.2** shows black HDPE piping threaded through a continuous one-pass trencher just prior to starting a section of biowall. For the trencher shown, a pilot trench is first excavated to 10 to 12 feet below grade by a backhoe. The trenching rig is then set up at the starting position, with the HDPE pipe extending out from the trench box. As the trenching chain and trench box are lowered into the pilot trench to start excavation, the pipe is left exposed at the ground surface and is continually fed from the back of the trencher. At the end of the trench, the pipe is again left exposed at the ground surface.

Similar to the need to develop monitoring wells, experience has shown that horizontal piping installed at the base of the biowall trench may require some form of development (surging and pumping) to enhance the ability to extract groundwater or to inject an emulsified vegetable oil substrate. When piping is installed in an open trench or bioreactor using conventional excavation equipment, it is therefore advisable to install gravel (only) or filter fabric around the perforated piping to prevent clogging or sedimentation of fine materials.

Surface piping completions are constructed similar to flush-mounted well boxes with end caps. A second HDPE or slotted PVC distribution pipe may be installed above the mulch mixture backfill for injection purposes. The surface piping is usually placed on the hydraulically upgradient side of the biowall so that monitoring wells can be installed within the biowall without damaging the pipe.

A synthetic geotextile material is typically used for the biowall surface completion to prevent soil backfill from fouling the biowall mulch mixture or distribution piping. Soil backfill using biowall soil is then backfilled from 3 to 4 feet bgs to the surface. Alternately, imported clay soil may be backfilled and compacted at the trench surface. An additional 1- to 2-foot soil mound is commonly placed over the biowall alignment to compensate for future settlement.

4.3.3 Bioreactor Installation

Installation of bioreactors follows similar methods to a biowall, although the entire excavation is usually open prior to installing the sand/mulch mixture and piping. The mixture may be installed in lifts of 1 to 3 feet, with a backhoe or front end loader used to compact the mixture once it rises above the water table. Piping for recirculation is typically installed close to the top of the excavation, and overlain with pea gravel or geotextile fabric to prevent clogging. The open nature of the excavation allows for better visual inspection of the bioreactor installation.

4.3.4 Construction Quality Control Plan

A construction QC plan is useful to ensure that construction specifications are met and that construction proceeds in a timely and efficient manner. Examples of construction QC activities for a biowall installation are listed in **Table 4.2**; similar examples may be applied to bioreactor installations. QC activities include ensuring that 1) all site preparation activities are completed prior to beginning biowall installation, 2) equipment is operating properly and safely, 3) the mulch mixture has been prepared properly and backfill material availability will not slow construction, 4) installation of monitoring wells has been completed properly, 5) health and safety monitoring is performed, and 6) as-built records of the biowall are maintained. Photo or video documentation is also very useful for preparing construction completion reports. These inspection activities will ensure that the biowall is installed in accordance with the engineering design and that all components of reporting can be fully met.

4.3.5 Inspection and Testing Requirements

An effective QA/QC program depends on thorough monitoring of all construction activities. This is most effectively accomplished by observation and documentation during all phases of construction. Documentation may consist of project submittals, daily QC inspection reports, weekly QC summary reports, non-conformance and corrective action reports, design and specification clarifications or modifications, photographic records, observation and testing data sheets, as-built documentation, and a summary report.

Daily QC Reports may include the following information:

- Site identification;
- Estimated volume of excavated material staged or shipped offsite during the day;
- Weather conditions;
- Narrative description of inspections, tests, and sampling; and
- Narrative description of work performed, problems encountered, and corrective measures taken.

Table 4.2
Examples of Construction Inspection Activities for Biowall Construction

Construction Inspection Activity	Method	Frequency	Acceptance Criteria
Demarcation of Biowall	Site Survey – Survey in alignment and mark with grade stakes	Once along biowall alignment prior to construction of the section.	Establish grade stakes along the designed biowall alignment according to the design drawings. Grade stakes should be placed at the start and termination of each segment, at 50-foot intervals along each segment, at 20-foot intervals along curves, and at any change in biowall direction.
Demarcation of Monitoring Wells	Site Survey – Survey in monitoring well locations	Twice: 1) Prior to installation of hydraulically upgradient and downgradient wells. 2) Prior to installation of wells in biowall.	Establish grade stakes at well locations according to the design drawings.
Dig Permit Acceptance	Obtain dig permits from facility engineer	Review prior to each excavation, biowall segment, or monitoring well installation.	Confirm that each location has been cleared for intrusive work and all utilities are clearly marked.
Equipment Examinations (Drilling and Earthwork)	Visual	Upon arrival at site and daily thereafter.	Determine that equipment type and size conform to project specifications and record information in field book. Determine that equipment conforms to OSHA safety requirements. Determine that equipment is in working order and is not leaking oil or fuel.
Mulch Material Stockpile	Visual	Once prior to commencing biowall installation.	Confirm that 50% of the required mulch material is on site and homogenized.

(continued)

Table 4.2 (Continued)
Examples of Construction Inspection Activities for Biowall Construction

Construction Inspection Activity	Method	Frequency	Acceptance Criteria
Mulch Mixture Delivery	Visual	Daily	Review the individual materials (*e.g.*, mulch, sand, and cotton gin trash) as they arrive on site to ensure that they meet the design specifications. Collect samples for analysis if specified.
Mulch Mixture Examination	Percentage of Organics (bulk density)	Every 100 feet along the alignment.	Perform testing of the mulch mixture as described in the field sampling plan (FSP).
Drilling Equipment Decontamination	Visual	Prior to installation of each monitoring well.	Confirm that the augers and other equipment that will be placed in the auger hole have been decontaminated.
Construction Methods Observation	Visual	During start-up and construction.	Ensure that the methods conform to standard construction practices and the worker safety is always a primary consideration.
Air Monitoring	Photoionization detector in worker breathing zone with appropriate detector lamp	During start-up and construction.	Readings below criteria established in the Health and Safety Plan (HASP).
Staging of Mulch Mixture	Visual	During start-up and construction.	Ensure that sufficient mulch mixture is stockpiled along the biowall alignment to allow at least one day of trenching operation. Ensure that haul routes are clearly understood and hauling operations minimize impact to facility traffic patterns.
Depth of Trenching	Visual/Measurement	Every 10 feet of biowall.	The depth of the biowall should be maximized and recorded in the field log for each 10-foot station increment.

(continued)

Table 4.2 (Concluded)
Examples of Construction Inspection Activities for Biowall Construction

Construction Inspection Activity	Method	Frequency	Acceptance Criteria
Trenching Spoils	Visual	Every 10 feet of biowall.	A description of the soil cutting composition, relative moisture content, and VOC headspace will be recorded in the field note book for each 10-foot station increment.
Thickness (width) of Trenching	Visual	Every 10 feet of biowall.	The thickness of the biowall should be maximized and recorded in the field log for each 10-foot station increment.
Backfill of Trenching	Visual	Every 10 feet of biowall.	The mulch mixture should be placed in the biowall to at least 3 feet above the high water table elevation, or as specified in the final design.
Distribution Piping	Visual	During construction.	The distribution piping installed on the bottom of the biowall should be laid in as straight of a line as possible down the center of the biowall. The distribution piping installed near the top of the biowall should be laid in as straight of a line as possible along the hydraulically upgradient side of the biowall.
Geotextile Placement	Visual	Daily during biowall backfill.	Confirm that a geotextile layer meeting specifications was placed over the mulch and distribution piping prior to backfill (top piping only).
Site Security	Visual	Daily during construction.	Confirm that any open biowall is fenced off and the work area perimeter is secure.
Soil Disposal	Visual	Daily during construction.	Confirm that soil analyses indicate that biowall spoils can be disposed of on site. Prevent soil from accumulating on roadways. Ensure soil is spread properly, or disposed per the residuals management plan.

Weekly QC Summary Reports may include the following information:

- Date, project name, and location;
- Summary of construction-related activities;
- Summary of QC activities;
- Attached inspection reports;
- Test results;
- Volume of soil staged for disposal;
- Volume of soil shipped for disposal to other locations (*e.g.*, offsite, if necessary);
- Non-conformance reports;
- Non-conformance/corrective action tracking log; and
- Corrective action reports.

The project manager or field supervisor should review unresolved corrective actions and take appropriate measures to ensure that the corrective actions are completed on schedule. An inspection should be conducted to verify that the corrective action is resolved, and that the resolution is documented in the Daily and Weekly QC Reports.

4.3.5.1 Inspection and Testing of Mulch Mixture

Materials for biowall construction should be inspected and volumes confirmed prior to homogenization, and the mulch mixture should be inspected during and following homogenization. Prior to mixing the individual components of the mulch mixture, and the volumes of the individual staging piles should be confirmed. It may be useful to segregate piles of individual components according to the correct volume and ratio of the final mulch mixture for each biowall segment. This allows the subcontractor to mix the components without having to track how much of an individual component has been added during mixing.

4.3.5.2 Inspection of Biowall Trench or Bioreactor Dimensions

Biowall trench or bioreactor cell dimensions should be continuously inspected during construction, and the extent of the biowall or bioreactor marked and surveyed upon completion. Visual inspection and tape measurements may be used to document that the full surface area has been installed. The depth of trenching or excavation may be confirmed by observing the depth to which the trenching equipment extends during construction. Continuous one-pass trenchers have visual indicators of the depth to which the trenching chain extends. For backhoe excavations, it may be useful to measure and mark the boom with the target depth prior to construction. Extension of the boom to the marked depth is visual confirmation that the target depth has been achieved.

4.3.5.3 Confirmation of Mulch Emplacement

Confirmation of mulch emplacement begins with documenting that the appropriate volume of the mulch mixture was installed in each biowall segment or bioreactor cell as designed. The volumes of staged piles of the mulch mixture should be confirmed prior to construction. An excess of substrate mixture during or after construction may be an indication that the trench dimensions were not to design, perhaps due to caving of the trench walls.

Visual inspection of the trenching operation provides insight as to whether the trench is being properly excavated and the mulch mixture is being placed without caving or sloughing of the trench walls. A video camera mounted on the back of the excavation equipment or lowered into the trench using a boom may be used to document media flow. This reduces worker exposure to hazardous conditions near the trench. A review of visual observations or video recordings may be used to identify potential problem areas and guide the selection of locations to conduct confirmation sampling.

Confirmation of mulch mixture emplacement following construction may be performed by drilling and coring select locations along the biowall alignment or within a bioreactor cell. This is readily accomplished when monitoring or injection wells are being installed within the biowall trench or bioreactor cell. Continuous cores should be collected and inspected for the presence of native soil (*i.e.*, caving or bridging) and for uniformity of the mulch mixture (*i.e.*, segregation of mulch and sand/gravel). Additional borings may only be warranted if inspection of the trenching operation or an excess of biowall backfill material indicates difficulty in emplacing the mulch mixture. It is generally not practical to "re-install" the mulch mixture. Where construction is not thought to be adequate, either additional biowall segments may be installed parallel to the initial trench section, or a slow-release fluid substrate may be injected to fill "gaps" in the treatment zone.

4.3.5.4 As-Built Specifications

The site engineer should establish and maintain a set of project drawings on site for the purpose of noting changes to design specifications. The need to address design and specification changes may arise, often resulting in a change in the subcontractors scope of work. In such cases, the project manager should notify the site owner or RPM. A field change in design, specification, or scope that may potentially impact the performance of the biowall or its cost should be approved before it is implemented.

Changes to project drawings are noted in red ink or pencil and referenced to changes approved by the site engineer. New drawings are added to the set if required for major or extensive changes. A working copy of all as-built drawings, as well as copies of all field changes, change orders, notes, sketches, and memoranda should be available for reference in the project field office. At the completion of the construction effort, field drawings are drafted into final as-built drawings.

4.4 MONITORING NETWORK INSTALLATION

Design of an appropriate monitoring network is described in **Section 3.11**. Monitoring networks should be completed as soon as possible after biowall or bioreactor construction is completed, but preferably after any site restoration that requires heavy equipment to prevent potential damage to surface completions. True "base-line" conditions within the biowall or

bioreactor may not be practical to obtain; these monitoring locations may not be completed for several days to a week or more until construction and site restoration are completed. Monitoring locations upgradient of the biowall or bioreactor may be used to establish background conditions.

Monitoring wells are usually installed using conventional drilling methods, such as hollow-stem auger. For some sites, monitoring points may be installed using direct push methods. Care should be taken when installing monitoring wells or points within the biowall or bioreactor not to damage any piping installed during construction.

4.5 FOLLOW-UP INSPECTION AND SITE RESTORATION

Field inspection for well installation, biowall as-built locations, and site restoration activities are identified in **Table 4.3**. Inspection activities include observations to verify the final location of the constructed biowall or bioreactor, and that the site is graded according to design. Restoration activities may include re-seeding the site or replacement of utilities and roadways. A final site survey is used for confirmation of final as-built drawings.

Table 4.3
Follow-Up Inspection Activities for Biowall or Bioreactor Construction

Follow-Up Inspection Activity	Method	Frequency	Acceptance Criteria
Monitoring Well Installation	Visual	During construction of each monitoring well.	Well is installed at the location specified, total depth and screened intervals are completed as specified, surface completion performed, and well is labeled.
Location of Biowall Segments, Bioreactor Cells, and Monitoring Wells	Site Survey – Licensed or registered surveyor	Once after construction along biowall alignment or bioreactor cell, and monitoring well network.	Survey along the constructed biowall alignment or bioreactor cell and collect elevations. For biowalls, location and elevation should be surveyed at the start and termination of each segment, at 50-foot intervals along each segment, at 20-foot intervals along curves, at any change in biowall direction, and at any change in grade.
Site Restoration	Visual	Once along biowall alignment or bioreactor cell, and at the staging areas, after construction is completed.	Ensure that excavation spoils are properly disposed of. Ensure the biowall alignment or bioreactor cell is graded to specifications and seeded. Ensure that the staging areas are clean, graded flat, and seeded.

SECTION 5

RESIDUALS MANAGEMENT PLAN

5.1 TRENCHING SOIL

Trenching and excavation operations may produce several hundred to thousands of cubic yards of excavated soil that may potentially contain hazardous levels of contaminants. If trenching spoils must be transported off site for disposal it may render the biowall technology cost prohibitive relative to other *in situ* bioremediation techniques using direct injection. *In situ* bioreactors are typically installed in source areas where the excavation, handling, and treatment of the soil is already being implemented as a source reduction measure.

Transport and off-site disposal of excavated trench soil may render the biowall technology cost prohibitive relative to other in situ bioremediation techniques using direct injection.

In most cases the trench spoils will not pose a hazard if properly managed onsite. A residuals management plan is required to effectively manage excavated trench soil and other investigation-derived wastes.

Excavated trench soil is temporarily placed in a continuous pile or "windrow" along the biowall alignment during construction (**Figure 5.1**). Trenching results in a highly mixed and disturbed soil. Free water (*i.e.*, groundwater not entrained in the soil) is usually limited in conjunction with the excavated soil, although the lay down area should be designed to contain any free water. Laying the excavated soil on heavy plastic sheeting to prevent potential contamination of surface soil may be warranted (**Figure 5.2**).

Site characterization data may be available to determine the potential for excavated soil to pose a health hazard. In most cases confirmation sampling is required to determine final disposition of the soil. A quick turnaround time to characterize the soil is desired if the soil is to be used for site restoration. More rigorous staging requirements (*e.g.*, RCRA requirements for staging piles) may be required when trench soils are to be staged for a month or more.

5.1.1 Soil Screening Criteria

Most biowall systems are installed as downgradient biobarriers to intercept a groundwater contaminant plume, and are not installed directly in source areas. Therefore, the potential for excavated soil to present a hazard is proportional to the concentration of the contaminant in groundwater, its sorption potential, and the amount of organic carbon or sediments with an ionic charge (*i.e.*, clay) in the aquifer matrix. For example, perchlorate has a low potential for sorption (see **Table C.1B** in **Appendix C**), and should not accumulate within the aquifer matrix.

Figure 5.1 Laying Excavated Trench Soil in a Windrow Along the Biowall Alignment, Altus AFB, Oklahoma

Figure 5.2 Laying Excavated Trench Soil on Plastic Sheeting, Altus AFB, Oklahoma

Even for TCE, the probability for trench spoils to pose a health risk is low based on its physical and chemical properties (**Table C.1A**). For example, biowall soils were screened and analyzed for a full-scale biowall application at Altus AFB in Oklahoma, where concentrations of TCE in groundwater were on the order of 5.0 µg/L to greater than 10,000 µg/L. **Table 5.1** summarizes the analytical results of TCE in groundwater monitoring wells relative to the concentrations of TCE measured in trench spoils along the same biowall section. Comparison of concentrations of TCE in groundwater to TCE in soil indicates that groundwater concentrations less than 1,000 µg/L typically do not yield soil concentrations greater than 0.1 milligrams per kilogram (mg/kg). Note that exceptions may occur in soils high in clay or organic carbon content (*e.g.*, glacial till or peat deposits).

Table 5.1 Analytical Results for Trench Spoils, SS-17 Biowall System, Altus AFB, Oklahoma

Concentration of TCE in Groundwater (µg/L)[a/]	Concentration of TCE in Soil (mg/kg)[b/]	Number of Samples
5 - 100	0.0001 - 0.0059	13
100 - 1,000	0.0013J[c/] - 0.0126	28
1,000 - 10,000	0.001 - 0.120	25
> 10,000	0.10 - 2.36	23

[a/] µg/L = micrograms per liter

[b/] mg/kg = milligrams per kilogram

[a/] J flag indicates concentration is estimated

Based on regulatory criteria for disposal of soil at Altus AFB, soil with concentrations of TCE less than 0.1 mg/kg were used as biowall cover material and for site grading and restoration. Soil from the few locations where concentrations of TCE exceeded 0.1 mg/kg were segregated and staged for further confirmation sampling. Confirmation sampling yielded results less than 0.1 mg/kg TCE, and the soil was disposed at a sanitary landfill on Altus AFB.

Given the low probability of encountering contaminated soil at sites with low to moderate concentrations of TCE, excavated soils are typically not staged beyond the windrow in which they are placed during construction. For most applications, excavated soil can be used to backfill the trench and graded over the biowall excavation for site restoration. However, screening of the biowall soils should be conducted during biowall construction as a preventive measure in the event that contaminated soil is encountered.

5.1.2 Soil Screening and Analytical Testing

A soil sampling plan is useful for screening soils to determine final disposition. Excavated soil recovered during the trenching operation is stockpiled adjacent to the portions of the biowall from which it originated. A typical sampling protocol is to sample at a frequency of one sample per every 100 to 200 linear feet of biowall. The soil samples may be either discrete samples based on field observations, or composite samples. The locations of soil samples for CAHs should correlate to locations exhibiting elevated levels of volatile organic compounds (VOCs) based on screening headspace vapors with a photoionization detector (PID). PID headspace readings are typically collected every 10 to 25 linear feet of biowall, or when a change in the

physical characteristics of the excavated soil is observed (*e.g.*, change in lithology or stained soil).

Samples are typically collected using an EnCore™ sampling device and analyzed for VOCs using USEPA Method SW8260B. Samples should be collected within a few hours of excavation as each biowall section is completed. A sampling log should be kept and should include the completion time of the section of the biowall, results and locations of any field screening, and identification and time of the soil sample collected for laboratory analysis.

For sites with other contaminants, appropriate soil analytical protocols should be used (see **Table 6.2**). Perchlorate and explosive compounds are not VOCs, so screening with a PID may not be appropriate. In any event, the soil sampling procedures and analytical protocol should be included in the residuals management plan and approved by the appropriate regulatory agency prior to mobilization for biowall installation.

5.1.3 Soil Disposal

To date, all Air Force biowall projects have used excavated trench soil to cover the biowall excavation and to restore the site to a positive grade so that surface water does not collect over the biowall. Site access is restricted for many DoD facilities, which facilitates the use of excavated soil for site restoration. Public access may warrant special precautions and handling of excavated soil. Site restoration should include seeding and stabilization of the soil cover to prevent erosion and surface runoff.

Alternatively, soil may be managed in place if use of the soil is restricted. For example, soil could be land farmed by mixing in mulch and staging the soil mix in an area upgradient of the biowall system. This may be a suitable approach for both CAHs and perchlorate. Installing a clean soil berm around the soil pile can be used to contain any potential contamination associated with an accumulation of surface water. Any potentially contaminated water that leaches to groundwater during the soil treatment period will be treated by the biowall system as it migrates in a downgradient direction.

5.2 FLUIDS GENERATED DURING TRENCHING

A designated lay-down area for excavated soil adjacent to the biowall should be constructed in a manner that allows for the management of any free liquids that might be present during construction of the biowall trench. The lay-down area may be graded to slope toward the biowall to allow any potential free water to flow into the biowall where it is returned to the constructed treatment zone. A small soil berm may be warranted on the side of the lay-down area furthest from the biowall to prevent potential surface run off. A sheet of plastic, approximately 20 ft wide, may be placed under soil spoils in the lay-down area as a further precaution (**Figure 5.2**). Grading and berms in the lay-down area ensures that any free liquid that might be generated during excavation will flow across the plastic liner and back into the biowall.

Free water that does not drain back into the biowall excavation may be collected for disposal in a manner specified for well development or purge water. In instances where large amounts of water may be generated or the likelihood of pooling is high, an onsite mobile water treatment system may be commissioned to treat the water onsite. Treated water may be disposed to local

sanitary or storm water drains. Permitting and confirmation sampling are likely to be required in this case.

5.3 MANAGEMENT OF INVESTIGATION DERIVED WASTE

Investigation-derived waste (IDW) generated during construction of a biowall or bioreactor system includes soil generated during installation of groundwater monitoring wells, purge water generated during development and sampling of monitoring wells, equipment decontamination rinsate, and PPE used during sampling activities. Procedures to handle this IDW are typically established during site investigation activities.

Solid drill cuttings are containerized and samples submitted for characterization as a hazardous or non-hazardous waste. Soil cuttings that are non-hazardous may be mixed with non-hazardous excavated trench soil and spread or graded during site restoration. Hazardous soil must be handled in accordance with applicable regulatory criteria.

Wastewater generated during drill rig decontamination, monitoring well development, and monitoring well sampling may contain CAHs or other site-specific contaminants. Therefore, the wastewater should be containerized and characterized for proper disposal in accordance with applicable regulatory criteria.

SECTION 6

FIELD SAMPLING PLAN

A FSP describes the field sampling procedures and protocols to be performed during construction and monitoring of a permeable mulch biowall or an *in situ* bioreactor. This FSP includes test methods and analytical protocols useful for evaluating the performance of permeable mulch biowalls and bioreactors. The following guidance documents are also useful in the preparation of a FSP:

- *AFCEE Model Field Sampling Plan, Version 1.2* (AFCEE, 2002a).
- *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater* (USEPA, 1998a).

In addition to a FSP, a formal Sampling and Analysis Plan (SAP) also includes a laboratory Quality Assurance Project Plan (QAPP). The following guidance documents are useful in the preparation of a QAPP:

- *Quality Assurance Project Plan, Final Version 4.0.02* (AFCEE, 2005).
- *Data Quality Objectives Process for Hazardous Waste Site Investigations* (USEPA, 2000a).

The user is referred to the AFCEE QAPP (AFCEE, 2005) for detail regarding laboratory QA/QC procedures. The FSP discussed in this section of the protocol focuses on field sampling, field test methods, and analytical protocols commonly used during installation and monitoring of biowalls and bioreactors.

6.1 MONITORING STRATEGIES FOR A FIELD SAMPLING PLAN

Monitoring locations and test protocols are based on a strategy to document that the system has been installed and is operating as designed, as well as documenting that performance objectives have been attained. Monitoring strategies for biowalls and bioreactors are driven by site-specific performance objectives, O&M requirements, and the configuration of the system. Regulatory requirements also may dictate certain monitoring protocols and frequency.

System monitoring is generally conducted for three purposes:

1) ***Baseline monitoring*** for characterization of contaminant distribution and groundwater biogeochemistry prior to construction is conducted to provide a basis for system design and to define the baseline for comparison to performance monitoring data.

2) ***Performance monitoring*** is conducted after construction to evaluate the performance of the system with regards to achieving remedial objectives; and

3) ***Process monitoring*** is conducted to evaluate the need for system modifications (*e.g.*, substrate replenishment or other amendments) that may be required to optimize the performance of the system.

The test methods and analytical protocols for each type of monitoring may differ based on the monitoring objectives. Monitoring protocols and frequency should remain flexible throughout the project to 1) incorporate optional diagnostic analyses (*e.g.*, dissolved hydrogen or microbial characterization), 2) allow alteration of the sampling frequency or protocol in response to changing conditions, or 3) allow for elimination of parameters that are not providing useful information.

A FSP should be prepared and followed to maintain a high level of QA/QC during installation and monitoring of a biowall or bioreactor system. The FSP establishes field sampling protocols, including sample collection procedures, equipment and instrument use, sample preservation and storage, maintenance of field records, sample transport and chain-of-custody protocols, and decontamination procedures. The following sections describe common sampling methods and analytical protocols for biowall materials, soil, and groundwater.

6.2 BIOWALL MATERIAL SAMPLING AND QA/QC

Materials for a biowall system (*e.g.*, sand, gravel, and mulch) must be evaluated prior to procurement for suitability of use. Samples of the field-homogenized mixture should also be evaluated prior to placement in the biowall for QA/QC purposes.

6.2.1 Sampling of Biowall Materials

Samples of the bulk biowall materials should be obtained prior to procurement and delivery to the site to ensure that the physical and chemical properties of the materials are suitable for use in the biowall or bioreactor system. Two or three composite samples of the mulch mixture may be chopped or crushed (*e.g.*, in a blender) and screened to remove material greater than 0.25 inches in diameter. The samples may then be submitted for fiber analysis and analysis of essential nutrients including nitrogen, phosphorous, and potassium.

Properties of the mulch or compost material may also be characterized using test methods developed by the National Forage Testing Association (NFTA) or by the American Society of Agronomy (ASA) using certified laboratories and procedures (www.foragetesting.org or www.agronomy.org). These methods have primarily been developed for forage materials, primarily hay. These analyses are relatively inexpensive (an entire suite of analyses, including elemental and nutrient analysis, often costs less than $100/sample). The analyses provide useful information about the mulch, including the total available polysaccharide substrate content (*i.e.*, relative amount of cellulose and hemicellulose), inert material content (lignin), and other indicators that may predict the bioavailability of the organic carbon in the mulch (Jung, 1997). These methods are useful in comparing various sources of mulch or compost (Ahmad *et al.*, 2007a). An example of the analysis of fiber content of the mulch and cotton gin trash used at

Altus AFB, Oklahoma can be found in Ahmad *et al.*, 2007A, and in Shen and Wilson (2007, supporting data, summarized in **Table 3.2**).

Composite samples of sand or gravel may be collected, screened to remove material greater than 0.25 inch in diameter, and submitted for analysis of total or bioavailable iron. If geotechnical parameters for the sand and gravel material are specified (*e.g.*, less than 5 % fines as silt and clay), then analysis of grain size distribution is warranted. Analytical methods and the data use for these parameters are described in **Section 6.5**.

Biowall materials added to stimulate abiotic processes may include sources of sulfate or iron (**Appendix D**). Sulfate is usually procured in the form of gypsum powder or gypsum fertilizer pellets. The mass of sulfate per unit weight of these products may vary substantially, and usually it is sufficient to obtain the percentage of sulfate and other inert elements or compounds from the manufacturer. Ferric iron may be procured in the form of high-iron sand or iron ore. Analyzing sand mixtures from various sources for total and bioavailable iron (**Section 6.5**) is one practical method to locate a source of high-iron sand. The total iron content in iron ores is typically provided by the supplier, but it may be advisable to analyze for bioavailable iron to ensure that reduction of the iron will enhance the formation of reduced iron sulfides.

6.2.2 Biowall Mixture Batch Test

An optional bench-scale test may be run to evaluate the suitability of the mulch mixture for the bioremediation application. This is primarily to evaluate adverse conditions such as low buffering capacity and undesirable pH excursions. Samples of the mulch, sand, and site groundwater are obtained and mixed for a batch test to determine the pH and geochemical properties after an appropriate incubation period. The incubation period may extend for several weeks to allow the mixture to acclimate to anaerobic conditions. Based on field observations, highly anaerobic conditions are usually established within 4 to 6 weeks of biowall construction. For example, concentrations of methane within the OU-1 biowall at Altus AFB, Oklahoma were 7.9 mg/L and 8.8 mg/L at two sample locations within the biowall at approximately 4 weeks after installation (Table 1 in **Appendix F.2**), relative to background levels of less than 0.1 mg/L.

Once the batch mixture has acclimated, the water is carefully drained and sampled, and replaced with fresh groundwater. The batch mixture may then be allowed to incubate for a period corresponding to the anticipated residence time in the biowall or bioreactor, typically on the order of 1 to 2 weeks. This water is then sampled and analyzed. Groundwater may continue to be replaced if additional evaluation is desired. For example, the batch mixture could be allowed to incubate for another 4 to 6 weeks and the sampling repeated. Conducting multiple incubations and sampling events may provide additional insight into how the geochemistry of the mulch mixture may change over time.

To conduct the test, a sample of the biowall material (typically 50 percent by volume mulch and 50 percent by volume sand/gravel) is mixed and placed in a 1-quart mason jar to approximately three-quarters full, and filled with groundwater collected from the site to cover the mulch mixture. The headspace in the jar is purged with carbon dioxide, and the jar sealed with a lid and a one-way fermentation lock to release any carbon dioxide or biogenic gas produced. The batch mixture is allowed to incubate in a dark location. A duplicate or triplicate batch test is run for confirmation of test results.

All samples of the batch test groundwater should be analyzed before mixing and after the incubation period for pH, alkalinity, ORP, DO, nitrate, ferrous iron, manganese, sulfate, sulfide, and carbon dioxide using a portable Hach® colorimeter or titration field test kits. The results of these tests are used to determine whether the biowall materials are suitable for use (*i.e.*, no adverse geochemical conditions are observed), or whether alternative materials or additional amendments are required (*e.g.*, crushed limestone to buffer pH to above 6.5). Analysis of contaminant concentration is optional; the purpose of the batch test is to determine the biogeochemical properties of the mulch mixture with a low-cost method.

6.2.3 Biowall Mixture Column Studies

More sophisticated column studies may also be conducted to determine the geochemistry of the mulch mixture and its hydrogeologic properties (*e.g.*, effective porosity and hydraulic conductivity). Analysis of influent and effluent contaminant concentrations may be measured to provide information on degradation rates and the residence time required for treatment. However, the column should be allowed to acclimate over a prolonged period of several weeks to months to obtain representative results. An example of a column study for chlorinated ethenes is described by Shen and Wilson (2007), an example for perchlorate is described by Perlmutter *et al.* (2001), and an example for RDX is described by Ahmad *et al*. (2007a).

In many cases the batch test described above is suitable for selection of materials that are local to the site, and the time and cost to conduct column studies may not be warranted. The use of column studies is typically not required or conducted as a routine procedure for design of a full-scale biowall or bioreactor system. Because a number of case studies have been reported, a better approach may be to extract relevant first-order decay constants for TCE and related contaminants from past case studies using the approach described in Ahmad *et al.* (2007b).

However, column studies may be warranted in situations where degradation pathways or kinetics are poorly understood, or where co-contamination exists. For example, suitable materials and appropriate quantities required to stimulate biogeochemical transformation of CAHs has not been fully explored. A column study, such as conducted by Shen and Wilson (2007), provides important data that cannot be extracted from the simplified batch test described above. An example might be a mass balance of sulfate and sulfide that can be used to correlate the observed degradation rate of CAHs to the production of iron monosulfides. Until further research and experience is gained in designing a biowall or bioreactor to stimulate abiotic degradation of CAHs, it is likely that the cost and time required to conduct a column study of potential backfill materials is worth the effort to reduce the risk of poor performance in the field.

6.2.4 Mulch Mixture Construction QA/QC

Throughout the biowall installation, the mulch mixture should be checked for proper consistency. Based on the analysis of the individual mulch components, a prescribed volumetric mulch mixture ratio is targeted, typically 50 percent mulch and 50 percent sand/gravel by volume. An example of a homogenization requirement for the backfill mixture is that the average percentage of organic material (by volume) in the total mixture ranges between 40 percent and 60 percent of the total sample volume. An example of a field QC procedure is described as follows.

The mulch mixture QC is performed by first collecting, settling, and weighing 2.5 gallons of mulch and 2.5 gallons of sand/gravel to obtain a bulk density for each material. These samples are then thoroughly mixed in a 5-gallon bucket to create a simulated QC mixture, agitated to settle the contents, and the volume and weight of the mixture is measured to calculate a bulk density of the simulated QC mixture. The combined mixture will have a volume less than 5.0 gallons as the sand fills the void space within the mulch. The volume and density of the simulated mixture are used to determine a volume loss coefficient associated with the homogenization process and to determine a target density for the field homogenized backfill material. A maximum and minimum density is similarly determined that corresponds to an average organic content of plus or minus 10 percent organic material by volume.

Representative samples of the field homogenized mulch mixture are then collected as it is mixed in the field. The samples are settled in a 5-gallon bucket, and the sample volume measured and weighed to determine a bulk density. If the variation of organic composition (determined by bulk density) of the mulch mixture after homogenization is greater than plus or minus 10 percent of the average organic composition, the mulch distribution in the mixture is corrected by additional homogenization and/or addition of mulch or sand to appropriate batches of the mixture until all portions of the mixture in a particular batch are compliant with QC requirements.

This process is typically conducted for every 25 to 50 cubic yards of mulch mixture, or whenever the physical characteristics of the bulk materials change. Examples of a change in the bulk materials may include a change in the amount of fines in the mulch or sand/gravel, or a change in moisture content (*e.g.*, during or following a rain event). The sensitivity and accuracy of the test method is most likely to be influenced by changes in moisture content. Visual inspection of the individual components and the final mixture should be conducted to ensure the test method is being properly applied.

Care should be taken when placing the mulch mixture in an open excavation filled with water. The mulch mixture will tend to separate if it is allowed to settle through a column of water due to density differences between the mulch, sand, or gravel materials. Continuous chain trenchers employ a trench box where there is a constant column of mulch being forced under gravity into the trench as it is being excavated. In this case there is minimal separation of the mulch mixture, as it is never allowed to settle through a column of standing water.

Where conventional construction techniques are used to place the mulch mixture in an open excavation, several methods may be employed to reduce the potential for separation of the materials. These include 1) shredding the mulch to a finer size and pre-hydrating it to increase its density, 2) lowering the mulch mixture in a large backhoe bucket to the base of the excavation before emptying the bucket, and 3) starting at one end of the excavation, bring the mulch to above the water table and then use a backhoe bucket to “push” the bulk mulch mixture down the face of the backfill as the mulch mixture is added.

6.3 FIELD SAMPLING PROCEDURES FOR SOIL AND MULCH

Sampling of native soil may be conducted during drilling for installation of groundwater monitoring wells. Sampling of biowall materials after biowall or bioreactor installation may be conducted to evaluate the chemistry and mineralogy of the material to determine the potential for

biogeochemical transformation of CAHs. Drilling may be accomplished using hollow-stem auger drilling, direct-push techniques, or other methods suitable for site conditions (*e.g.*, hand augers or backhoes for shallow applications). Soil sampling should be conducted by qualified scientists and technicians who are trained in sampling procedures, records documentation, and chain-of-custody procedures. Proper decontamination practices should be employed.

6.3.1 Borehole Advancement

During borehole advancement, soil samples for visual description are collected to identify the depths of significant stratigraphic contacts or other soil properties, with particular attention to potential zones of preferential groundwater flow. Soil samples are typically collected using split-spoon samplers from select borehole locations at approximately 5-foot intervals. A portion of each sample is used to measure the total ionizable VOC concentration in soil headspace using a PID. When drilling to collect samples from within the biowall or bioreactor, samples should collected to identify the consistency of the mulch mixture and the presence of any mineral staining (indicating the presence of reduced metal sulfides).

The field scientist is responsible for maintaining a detailed descriptive log of all subsurface materials recovered during drilling, recording field measurements, collecting soil samples, and properly labeling and storing samples. A boring log form is completed with a descriptive log that contains:

- Sample interval (top and bottom depth) and sample recovery;
- Presence or absence of contamination (*e.g.*, staining, odor, or elevated headspace screening readings);
- Soil description of the target sampling interval, including relative density, color, major textural constituents, minor constituents, porosity, relative moisture content, plasticity of fines, cohesiveness, grain size, structure or stratification, relative permeability, and any other significant observations; and
- The depth of lithologic contacts and/or significant textural changes measured and recorded to the nearest 0.5 foot, if present within the sampling intervals.

At select borehole locations, samples of native soil may be collected from the screened interval (*i.e.*, below the water table), and submitted to a fixed-base laboratory for analysis of total organic carbon (TOC) or fraction organic carbon (f_{oc}). This data is typically used to estimate retardation factors for contaminant transport.

6.3.2 Sampling for Mineral Speciation

Of particular interest in evaluating degradation processes in permeable mulch biowalls or bioreactors is biogeochemical transformation, in which chlorinated solvents are degraded abiotically by reactive iron sulfide minerals formed by, at least in part or indirectly from, anaerobic biological processes (*e.g.*, Butler and Hayes, 1999 and 2000; see **Section B.4.4** in **Appendix B** for further description of the process). Soil samples for analyses of iron, manganese, and sulfide may be collected from soil or biowall boreholes and analyzed for the following (See methods in **Section 6.4**):

- Acid Volatile Sulfide (AVS);
- Chromium Extractable (or reducible) Sulfide (CES);
- Bioavailable Iron;
- Weak Acid Soluble Ferric and Ferrous Iron (WAS-Fe^{2+} and WAS-Fe^{3+}), and Weak Acid Divalent Manganese (WAS-Mn^{2+}); and
- Strong Acid Soluble Ferrous and Ferric Iron (SAS-Fe^{2+} and SAS-Fe^{3+}), and Strong Acid Soluble Divalent Manganese (SAS-Mn^{2+})

Because these samples measure reduced states of iron and manganese minerals, they require special handling and preservation. Sample collection and preservation techniques are described by Wilkin (2007), AFCEE (2002b), and Kennedy *et al.* (1999). The following are sample protocols that may be followed for these samples. One is for large volume samples collected using brass, stainless steel, or acetate core sleeves; one is for smaller volume samples collected from discrete core samples; and one is for collecting frozen core samples using liquefied nitrogen. Experience has shown that the first two methods may not work well for loose and porous mulch and sand in a biowall. Very fine iron sulfide precipitates often flow out of the sample with groundwater as the sample is recovered to the surface. If the sample is dewatered during recovery, likely much of the AVS and CRS is lost with the water. In this case, the frozen core method is a better alternative.

Other methods may be employed. For example, piston-style or clam-shell core barrels may be used to extract cores under a vacuum to prevent loss of soil and groundwater. Samples may also be homogenized or processed for shipping in an anaerobic glove box in the field. The primary objective is to obtain representative samples with as little disturbance or oxidation as possible. Appropriate health and safety procedures should followed when using dry ice and compressed or liquid gas.

6.3.2.1 Sampling Procedure for Direct-Push, Split Spoons, or Core Barrels with Core Liners

Equipment Required

- Core liners, preferably 6- to 12-inch brass or stainless steel.
- Core-liner end-caps.
- Knife or saw (for plastic sleeves) for cutting core into sections.

Sampling Procedure

- Use core liners and bring a liner containing the target sediment to the surface.

- Cut or separate the section of the core sleeve from which the sample is to be collected to a length of 12 inches (1- to 2-inch core liners) or 6 inches (3- to 4-inch core liners), cap immediately, wrap twice in plastic wrap, and tape.

- Seal the sample sleeves in zip lock bags (2X) and place in cooler with dry ice to freeze.

Samples should remain frozen until delivered to the laboratory. Ship samples on dry ice. Do not fill with groundwater to avoid breakage or splitting of the core liner from freezing.

6.3.2.2 Sampling Procedure for Split Spoons or Core Barrels without Core Liners

Equipment Required

- Compressed nitrogen gas and regulator.

- 5 milliliter (ml) disposable syringes with the ends cut off.

- 40-ml volatile organic analysis (VOA) bottles with impermeable caps.

- Valves and flexible hoses to fit regulator, and bottle rack for purging air from VOA bottles.

- Dry ice, freezer bags, bubble wrap, and cooler.

Sampling Procedure

- Set up the nitrogen tank, regulator, and purge apparatus.

- Label two VOA bottles per sample analysis, remove caps, and begin purging vials with nitrogen. Keep bottles upright with thin tubing inserted to the bottom of the bottle.

- Bring the split-spoon or core sampler containing sediment to the surface.

- Immediately upon opening sampler, identify a representative sample interval and collect a sample by inserting the pointed, open end of a 5 ml disposable syringe.

- Transfer the sample to one of the VOA bottles, and collect a second sample to add additional sample volume to the VOA bottle.

- Immediately return the VOA bottle to the purging rack, insert the nitrogen purge tube to the bottom of the vial (as far as practical) and resume purging the bottle.

- Repeat procedure with second VOA bottle.

- After purging sample bottles, replace caps.

- Wrap bottles in bubble wrap, seal in zip lock bags (2X), and place in cooler with dry ice to freeze.

Samples should remain frozen until delivered to the laboratory. Ship samples on dry ice. Do not force sample into the VOA bottles or fill with groundwater, sample should be loose to avoid breakage of bottles due to expansion during freezing.

6.3.2.3 Sampling Procedure for In-Situ Frozen Biowall Samples

Equipment Required

- 1.5- to 2.25-inch outside-diameter (OD) direct push rods with O-rings and a fixed, solid drive tip. The drive tip must have a diameter slightly larger than the drive rod OD to create a shoulder to retain the frozen samples.
- 1/4- or 3/8-inch diameter copper tubing, depending on drive rod inside-diameter (ID).
- Liquid nitrogen "sprayer" consisting of an 18-inch length of the above copper tubing with the bottom end crimped closed, the top fitted with a standard compression fitting, and 1/16- to 3/32-inch holes drilled along the length (**Figure 6.1**).

Figure 6.1 Nitrogen Sprayer from Copper Tubing to Inject Liquid Nitrogen
(photos used with permission of the USEPA NRMRL/GWERD)

- Liquid nitrogen in a bottom-discharge cryogenic liquid container (dewar). Approximately 15 to 20 liters of liquid nitrogen is required per sample.
- Rock hammer.

- Specialized personal protective equipment for handling liquid nitrogen, for example cryogenic gloves and face shields.

Sampling Procedure

- Drill through the soil overburden and to approximately 1-foot above the top of the shallowest sample interval with minimum 8-inch OD augers when using 1.5-inch OD push rods, and minimum 10-inch OD augers for larger OD push rods.

- Push the drive rods to the bottom of the sample interval. Care should be taken to center the drive rods in the boring to facilitate sample retrieval.

- Insert the nitrogen sprayer down inside of the push rods to the bottom.

- Apply liquid nitrogen for approximately 5 to 10 minutes to create an approximately 2-inch thick layer of frozen biowall material on the outside of the lead drive rod. Exact time required for nitrogen application depends on the diameter and wall thickness of the push rods, groundwater temperature, and rate of nitrogen application.

- Stop the flow of nitrogen, wait until any liquid nitrogen remaining inside the drive rods volatilizes, then immediately withdraw the drive rods with the attached, frozen sample (**Figure 6.2**).

Figure 6.2 Frozen Sample of Biowall Material Collected Using Liquid Nitrogen Method at Altus AFB, Oklahoma
(photo used with permission of the USEPA NRMRL/GWERD)

- Place the lead push rod with sample on a clean plastic sheet and remove the desired sample interval using a decontaminated rock hammer.

- Immediately place the frozen sample into a plastic bag, seal, wrap with tape, attach the sample label with clear tape, then place on dry ice.

- Samples must be withdrawn, removed from the drive rod, wrapped, and placed in a cooler containing dry ice before the sample thaws.

Samples must remain frozen until delivered to the laboratory. Ship samples overnight on dry ice with appropriate labeling on the shipping containers.

6.3.3 Soil Sampling of Excavated Biowall Soil

Soil samples for analyses of potential contaminants are collected from excavated biowall soils for waste characterization and determination of appropriate disposal requirements as described in **Section 5**. Excavated trench soils recovered during the trenching operation are stockpiled in windrows adjacent to the alignment of the biowall, corresponding to the location where the spoils originated.

Determination of soil sampling frequency and screening criteria are described in **Section 5**. Samples for VOC analysis are typically collected using an Encore® sampling device. In addition, a duplicate sample, a matrix spike (MS) sample, and a matrix spike duplicate (MSD) sample are collected for QA/QC purposes. Analytical methods and the types of sample containers, sample volumes, and methods of preservation are described and listed in **Section 6.5**. The trench spoils are then disposed of in accordance with the residuals management plan.

6.4 GROUNDWATER SAMPLING

Groundwater sampling should be conducted by qualified scientists and technicians who are trained in groundwater sampling, records documentation, and chain-of-custody procedures. All field equipment coming in contact with potentially contaminated soil or water, or used for well development or sampling should be decontaminated before and after use. Laboratory-supplied sample containers should be cleaned and sealed by the laboratory.

All equipment to be used for well development or sampling should be assembled and properly cleaned and calibrated (if required) upon arrival in the field. As required, field analytical equipment is calibrated according to the manufacturers' specifications prior to field use. This applies to equipment used for onsite measurements of DO, pH, specific conductance, ORP, and other field parameters.

All monitoring wells require development or purging prior to sampling. Development removes sediment from inside the well casing and flushes fines from the portion of the formation adjacent to the screen. Development may be accomplished using a bailer and a submersible pump. Development is usually continued until a minimum 10 casing volumes of water have been removed from the well and until pH, temperature, specific conductance, DO, and ORP stabilize. If wells bail or pump dry, alternate development criteria may need to be followed. Typical well stabilization parameters are listed in **Table 6.1**. If the water remains turbid, development will continue until the turbidity of the water produced has been stable after the removal of several additional casing volumes.

Table 6.1
Typical Stabilization Criteria for Water Quality Parameters

Parameter	Stabilization Criteria
pH	+/- 0.1
Specific Conductance	+/- 5%
Dissolved Oxygen	+/- 0.5 mg/L
Oxidation-Reduction Potential	+/- 20 mV
Temperature	+/- 1.0 degrees Celsius

Notes: mg/L = milligrams per liter; mV = millivolts.

A peristaltic or bladder pump is typically used for low-flow purging and sampling. The micro-purging method, per the USEPA low-flow protocol (USEPA, 1996), offers the advantage that the amount of water to be containerized, treated, or stored is minimized. The low-flow sampling method is based on the assumption that pumping at a low rate within the screened zone will minimize the mixing of casing volume water with the sample collected from the surrounding formation. Using the low-flow sampling method, stagnant water is purged from the mid-point of the saturated portion of the well screen prior to sampling. Dedicated HDPE tubing is used in the well, and dedicated silicone tubing used in the peristaltic pump heads. Dedicated bladders are used in bladder pumps, as appropriate. When reusing equipment that is decontaminated between sampling locations, groundwater sampling should generally proceed from the least-contaminated to the most-contaminated wells to reduce the potential for cross-contamination.

Purging is continued until pH, temperature, specific conductance, DO, and ORP stabilize (**Table 6.1**). A well purge record should be maintained for each monitoring well. A peristaltic or bladder pump may also be used to extract groundwater for sample collection. The extracted groundwater is transferred directly into the appropriate sample containers for laboratory analysis (**Section 6.5**). Samples may also be collected for further field analysis (**Section 6.6**) using portable field test kits. Samples for these analyses are collected in plastic bottles (*e.g.*, 500 ml Nalgene® bottles) with no headspace, placed on ice, and analyzed as soon as possible after collection.

6.5 LABORATORY ANALYSES

Laboratory analyses are performed on collected soil, biowall materials, groundwater, and surface water samples, as well as QA/QC samples. Analytical methods for soil and biowall materials are listed in **Table 6.2**, and methods for water samples are listed in **Table 6.3**. **Table 6.4** lists appropriate sample containers and preservation for both solid and water samples for fixed-base laboratory analyses. All analytical samples should be immediately packed on ice and shipped to the appropriate lab for analysis. An exception is soil samples for analysis of sulfide, manganese, and iron as described in **Section 6.3.2**.

Table 6.2
Soil and Backfill Material Analytical Protocol for Permeable Mulch Biowalls and Bioreactors

Analyte	Method/Reference (laboratory/field)	Data Use	Data Implications	Frequency of Analysis
Chlorinated Aliphatic Hydrocarbons (CAHs)	SW5035/SW8260B Purge-and-Trap and Extraction/ Volatile Organic Compounds by GC/MS (laboratory)	Data are used to determine the extent and degree of soil contamination, to estimate the sorbed contaminant mass present, and to determine the need for other source removal actions.	A continuing source of contaminant mass from sorbed or free-phase DNAPL must be taken into account in the design and life-expectancy of a biowall system.	Recommended for native soil if a contaminant of concern. Initial sampling in source area(s). May be required for characterization of trench spoils.
Perchlorate	E314.0 Ion chromatography (laboratory)	Data are used to determine the extent and degree of soil contamination	A continuing source of contaminant mass in soil must be taken into account in the design and life-expectancy of a biowall system.	Recommended for native soil if a contaminant of concern. Initial sampling in source area(s). May be required for characterization of trench spoils.
Explosives (TNT, RDX, HMX)	SW846 Method 8095 Gas Chromatography with Electron Capture Detector, or SW8330 High Performance Liquid Chromatography (laboratory)	Data are used to determine the extent and degree of soil contamination	A continuing source of contaminant mass in soil must be taken into account in the design and life-expectancy of a biowall system.	Recommended for native soil if a contaminant of concern. Initial sampling in source area(s). May be required for characterization of trench spoils.
Pesticides and Herbicides	SW8081A Organochlorine Pesticides by Gas Chromatography, and SW8151A Chlorinated Herbicides by GC (laboratory)	Used to determine potential for contamination from mulch and compost materials. Most pesticides and herbicides will break down during staging and composting prior to installation.	Precautionary measure to prevent cross-contamination to groundwater. May be required by regulatory community.	Optional, for mulch and compost only. During procurement, prior to staging of biowall materials.
Nitrate as Nitrogen	SW9056 or E300.0 Ion Chromatography (laboratory)	Nitrogen is an essential nutrient for microbial growth.	A mulch mixture low in nitrogen may require amendment with a high nitrogen material. A long-lasting source of nitrogen is preferred.	Optional, for mulch and compost only. Prior to procurement and staging of biowall materials.

(continued)

Table 6.2
Soil and Backfill Material Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analyte	Method/Reference (laboratory/field)	Data Use	Data Implications	Frequency of Analysis
Total Kjeldahl Nitrogen (TKN)	E351.1 Automated Colorimetry or SM4500-NorgB - Macro-Kjeldahl Method (laboratory)	TKN is the sum of ammonia and organic nitrogen. Nitrogen is an essential nutrient for microbial growth. Alternative method.	A mulch mixture low in nitrogen may require amendment with a high nitrogen material. A long-lasting source of nitrogen is preferred.	Optional, for mulch and compost only. Prior to procurement and staging of biowall materials.
Acid Detergent Fiber (ADF)	NFTA Method 4.1 or similar, by refluxing or by Near Infrared Reflectance Spectroscopy (NIRS) (laboratory)	ADF measures portion of the mulch cell wall that are made up of cellulose and lignin.	In combination with ADL, provides a measure of the percentage of cellulose in mulch or compost. The greater the percentage of cellulose, the more readily degradable the mulch mixture will be.	Optional, for mulch and compost only. Prior to procurement and staging of biowall materials.
Neutral Detergent Fiber (NDF)	NFTA Method 5.1 or similar, by refluxing, amylase procedure, or by NIRS (laboratory)	NDF measures the total fiber content of the mulch cell walls, which is comprised of the ADF fraction plus hemicellulose. Subtracting ADF from NDF provides a percentage (by weight) of hemicellulose.	In combination with ADF, provides a measure of the percentage of hemicellulose in mulch or compost. Hemicellulose is more degradable than lignin.	Optional, for mulch and compost only. Prior to procurement and staging of biowall materials.
Acid Digestible Lignin or Acid Detergent Lignin (ADL)	Analysis of ADF fraction using sulfuric acid or potassium permanganate digestion. (laboratory specialty method)	ADL measures the lignin content of the mulch cell walls. Subtracting ADL from ADF provides a percentage (by weight) of cellulose. Alternate methods include acid detergent lignin and Klason lignin (Jung *et al.*, 1999).	The greater the percentage of lignin, the less degradable the mulch mixture will be.	Optional, for mulch and compost only. Prior to procurement and staging of biowall materials.
Potassium and Phosphorous	SW6010B Inductively Coupled Plasma-Atomic Emission Spectrometry or E365.1 (Phosphorous, laboratory)	Potassium and phosphorus are essential nutrients for microbial growth.	A mulch mixture low in potassium or phosphorous may require amendment. A long-lasting source potassium or phosphorous is preferred.	Optional, for mulch and compost only. Prior to procurement and staging of biowall materials.

(continued)

Table 6.2
Soil and Backfill Material Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analyte	Method/Reference (laboratory/field)	Data Use	Data Implications	Frequency of Analysis
Fraction of organic carbon (f_{oc})	SW9060A Total Organic Carbon modified for soil matrix (laboratory)	The fraction of organic carbon in the aquifer matrix is used to calculate retardation factors for dissolved contaminant transport and to estimate the amount of contaminant mass sorbed to the aquifer matrix.	A large proportion of contaminant mass may be sorbed to the aquifer matrix.	Recommended for native soil.
Grain Size Analysis	ASTM D-422 quantitative determination of the distribution of particle sizes in soils (geotechnical laboratory)	Indication of aquifer permeability and pore throat size. Also used to determine amount of fines in biowall backfill material.	The permeability of the biowall backfill material should be an order of magnitude or more higher than the surrounding formation.	Recommended prior to procurement and staging of biowall materials.
Major Cations (Fe, Mn, As, Ca, Mg, Na, K)	SW6010B Inductively Coupled Plasma-Atomic Emission Spectrometry, and SW6020 Inductively Coupled Plasma-Mass Spectrometry (laboratory)	Iron and manganese may be significant electron acceptors. Other cations may be monitored for degradation of secondary water quality or used for geochemical modeling.	High levels of iron may be suitable for stimulating biogeochemical transformation through the formation of reduced metal sulfides. Anaerobic degradation processes may be sensitive to geochemical conditions.	Optional prior to procurement of materials, or after installation and acclimation of the reaction zone.
Major Anions (Cl^-, SO_4^-, NO_3^-, CO_3^-, HCO_3^-, Br^-)	E300.0 or SW9056 Inorganic Anions by Ion Chromatography (laboratory)	Nitrate and sulfate may be significant electron acceptors. May be used for geochemical modeling.	Anaerobic degradation processes may be sensitive to geochemical conditions.	Optional prior to procurement of materials, or after installation and acclimation of the reaction zone.
Biologically Available Iron (Fe^{3+})	Laboratory specialty method by New Horizons (laboratory)	Bioassay with quantification of bioavailable solid-phase ferric iron (Fe^{3+}) that is a native electron acceptor. Also an indicator of a source of ferric iron for formation of iron sulfides.	Recommended for clastic sediments with potential for significant iron concentration, and for biowall and backfill material.	During site screening for native soil, and during procurement for biowall backfill material (sand and gravel).

(continued)

Table 6.2
Soil and Backfill Material Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (concluded)

Analyte	Method/Reference (laboratory/field)	Data Use	Data Implications	Frequency of Analysis
Weak Acid Extractable Iron and Manganese	Laboratory specialty method - Microseeps SOP-WC43/WC20; or AFCEE, 2002b and Kennedy *et al.*, 1999.	Approximation of bioavailable ferric iron and biogenic ferrous iron.	Optional method to biologically available iron and manganese via the bioassay method above.	Optional during site screening for native soil and for biowall backfill material (sand and gravel) during procurement.
Strong Acid Extractable Iron and Manganese	Laboratory specialty method - Microseeps SOP-WC43/WC20; or AFCEE, 2002b and Kennedy *et al.*, 1999.	Approximation of total iron an manganese.	Recommended for clastic sediments with potential for significant iron and manganese concentrations, and for biowall sand backfill material.	Optional during site screening for native soil and for biowall backfill material (sand and gravel) during procurement.
Acid Volatile Sulfide (AVS)	Laboratory specialty method - Microseeps SOP-WC43/WC03; or AFCEE, 2002b and Kennedy *et al.*, 1999.	Iron monosulfides formed under anaerobic conditions may be reactive with CAHs.	Indication of the amount of sulfide present as iron mono-sulfide minerals (FeS).	Optional after biowall installation.
Chromium Extractable Sulfide (CES)	Laboratory specialty method - Microseeps SOP-WC43/WC03; or AFCEE, 2002b and Kennedy *et al.*, 1999.	Indication of the valence state (reduced state) of sulfur species.	Following AVS extraction, and indication of the amount of sulfide present as elemental sulfur or divalent iron sulfide (Fe_2S)	Optional after biowall installation.
Carbonate Green Rust	Laboratory specialty methods.	Carbonate green rusts formed under anaerobic conditions may be reactive with CAHs.	Potential for abiotic degradation of CAHs by reactive carbonate green rusts.	Optional after biowall installation.

NOTES:
Analyses other than those listed in this table may be required for regulatory compliance.
1. "SW" refers to the *Test Methods for Evaluating Solid Waste, Physical, and Chemical Methods*, SW-846, USEPA, 3rd edition, 1986.
2. "ASTM" refers to the *American Society for Testing and Materials.*
3. "NFTA" refers to the *National Forage Testing Association.*

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors

Analysis	Method/Reference (Laboratory/Field)	Data Use	Performance Expectation or Implication	Recommended Frequency of Analysis
Chlorinated Aliphatic Hydrocarbons (CAHs)	SW8260B – Volatile Organic Compounds by GC/MS (laboratory)	Regulatory compliance for contaminants of concern. The values by which success of the remediation system will be measured.	CAHs and dechlorination products are typically expected to decline to less than regulatory compliance levels after migration or transport through the treatment zone.	Recommended for each sampling round.
Total Organic Carbon (TOC) or Dissolved Organic Carbon (DOC)	SW9060, EPA Method or E415.1 (laboratory)	Indicator of natural organic carbon present at site during baseline characterization and as an indicator of substrate distribution during performance monitoring. TOC/DOC concentrations greater than 10 to 20 mg/L are desired in the anaerobic treatment zone.	Stable or declining TOC/DOC levels less than 10 to 20 mg/L in conjunction with elevated levels of VOCs and alternate electron acceptors may indicate additional substrate is required to sustain the treatment zone.	Recommended for each sampling round.
Oxidation-Reduction Potential (ORP)	Direct reading meter, A2580B, or USGS, 1997 (field)	Highly reducing conditions are required for anaerobic dechlorination to occur. The ORP of groundwater provides data on whether anaerobic conditions are present. Used in conjunction with other geochemical parameters, ORP indicates which terminal electron accepting processes (TEAPs) predominate in an anaerobic environment and whether groundwater conditions are optimal for anaerobic biodegradation.	Field meter readings for ORP are typically measured against a silver/silver chloride (Ag/AgCl) reference electrode, and should remain less than -200 millivolts (mV), or less than 0 mV relative to a standard hydrogen electrode (SHE or Eh), within the treatment zone for anaerobic dechlorination of CAHs to occur. ORP readings higher than these levels, in conjunction with elevated levels of DO and the absence of TOC/DOC, may indicate that additional substrate is required to promote anaerobic dechlorination. Less reducing conditions may be required for degradation of perchlorate or explosive compounds.	Recommended for each sampling round. Typically measured at the well head using a flow-through cell to protect samples from exposure to oxygen.
Dissolved Oxygen (DO)	DO membrane electrode(E360.1) (field)	DO should be depleted in an anaerobic bioremediation system. DO less than 0.5 mg/L generally indicates an anaerobic pathway suitable for anaerobic dechlorination to occur.	DO concentrations greater than 1.0 mg/L, in conjunction with elevated levels of CAHs and the absence of TOC/DOC, indicate additional substrate may be required to promote anaerobic dechlorination.	Recommended for each sampling round. Typically measured at the well head using a flow-through cell.
Dissolved Oxygen (DO) (0 to 1 mg/L, or 0 to 10 mg/L)	ASTM D 5543-94 - Rhodazine D™ methodology, CHEMetrics Test Kit (field)	Same as above. Useful to check accuracy of field meter readings.	Same as above.	Use as a confirmatory analysis for membrane electrode method for wells with highly reducing groundwater.

(continued)

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Soluble Manganese (Mn[II])	Colorimetric Hach Method 8034 (field)	Manganese (IV) is an alternate electron acceptor for microbial respiration in the absence of oxygen and nitrate; reduction of manganese (IV) produces soluble manganese (II). Elevated levels of manganese indicate that the groundwater environment is sufficiently reducing to sustain manganese reduction and for potential anaerobic degradation processes to occur.	Elevated levels of manganese (II) may indicate a competing TEAP to anaerobic dechlorination of CAHs.	Recommended for each sampling round. Typically measured at the well head to protect samples from exposure to oxygen.
Ferrous Iron (Fe[II])	Colorimetric Hach Method 8146 (field)	Ferric iron is an alternate electron acceptor for microbial respiration in the absence of oxygen and nitrate; reduction of ferric iron produces ferrous iron. Elevated levels of ferrous iron indicate that the groundwater environment is sufficiently reducing to sustain iron reduction and for anaerobic dechlorination to occur.	Elevated levels of ferrous iron may indicate a competing TEAP to anaerobic dechlorination of CAHs.	Recommended for each sampling round. Typically measured at the well head to protect samples from exposure to oxygen.
Sulfate (SO_4^{2-})	E300.0A Ion Chromatography (laboratory) or Hach Method 8051 (field)	Sulfate is an alternate electron acceptor for microbial respiration in the absence of oxygen, nitrate, and ferric iron. Depleted concentrations of sulfate relative to background indicate that the groundwater environment is sufficiently reducing to sustain sulfate reduction and for anaerobic dechlorination to occur.	Sulfate levels less than 20 mg/L are desirable, but not required, for anaerobic dechlorination to occur. High levels of sulfate in conjunction with the absence of TOC/DOC indicate additional substrate may be required to promote anaerobic dechlorination.	Recommended each sampling round.
Methane, Ethane, and Ethene	Kampbell *et al.,* 1989 or lab SOP based on Microseeps AM20GAX (laboratory)	Elevated levels of methane indicate fermentation is occurring in a highly anaerobic environment and that reducing conditions are appropriate for anaerobic dechlorination of CAHs to occur. Elevated levels of ethene and ethane (at least an order of magnitude greater than background levels) can be used to infer complete anaerobic dechlorination of CAHs.	Methane levels greater than 1.0 mg/L are desirable, but not required, for dechlorination to occur. Methane levels less than 1.0 mg/L and the accumulation of *cis*-1,2-DCE, VC, or other less-chlorinated CAHs may indicate that additional substrate is required to create redox conditions suitable for reduction of these compounds. If elevated levels of ethene or ethane are not observed, potential accumulation of *cis*-1,2-DCE or VC should be monitored.	Recommended each sampling round. May require analysis by a specialty laboratory.

(continued)

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Alkalinity (total) as calcium carbonate	E310.1 (laboratory), or Hach Digital Titration Method 8203 (field)	Indicator of biodegradation and the buffering capacity of the aquifer (neutralization of weak acids). Used in conjunction with pH, an increase in alkalinity and stable pH indicates the buffering capacity of the aquifer is sufficient to neutralize metabolic acids produced by degradation of substrates.	Concentrations of alkalinity that remain at or below background in conjunction with pH less than 5 indicates that a buffering agent may be required to sustain high rates of anaerobic dechlorination.	Recommended each sampling round.
Carbon Dioxide	Microseeps AM25 (laboratory) or Hach Digital Titrator Method 8205 (field)	Carbon dioxide is a byproduct of both aerobic and anaerobic degradation. Elevated levels of carbon dioxide indicate microbial activity has been stimulated.	Indicator parameter only. In aquifer matrices with low alkalinity, aqueous carbon dioxide can accumulate and cause pH to decrease.	Optional.
Total Inorganic Carbon (TIC)	Laboratory Specialty Method	TIC includes aqueous carbon dioxide, carbonic acid and total carbonate alkalinity. The distribution is a function of pH. TIC expressed as milligrams Carbon per liter (mg C/L) can be calculated from field measurements of carbon dioxide and total alkalinity expressed as mg/L.	Increases in TIC relative to background concentrations provide an indicator of the areas with increased microbial activity.	Optional.
pH	Direct Reading Meter (field)	Biological processes are pH sensitive, and the ideal range of pH for dechlorinating bacteria is 5 to 9. Outside this range, biological activity is less likely to occur.	pH levels within a range of 5 to 9 are desirable. pH less than 5 indicates that a buffering agent may be required to sustain high rates of anaerobic dechlorination.	Recommended each sampling round.
Turbidity	Direct Reading Meter, E180.1 (field)	Measured prior to sampling to determine if sample filtration is necessary, or as a well purging stabilization parameter.	Low turbidity is desirable to reduce method interferences.	Optional.
Nitrate as Nitrogen	E300.0 Ion Chromatography (laboratory)	Nitrate is an alternate electron acceptor for microbial respiration in the absence of oxygen. Depleted levels of nitrate (relative to background) indicate that the groundwater environment is sufficiently reducing to sustain nitrate reduction.	Indicator parameter only. Nitrate levels less than 1.0 mg/L are desirable for anaerobic dechlorination of CAHs.	Optional. Recommended for each sampling round only if nitrate reduction appears to be a significant TEAP.
Nitrate/Nitrite as Nitrogen (total)	E353.2 Colorimetric, Automated, Cadmium Reduction (laboratory)	Same as above. Alternative to analyzing nitrate by Ion Chromatography methods. In most aquifer systems, concentrations of nitrate are naturally much higher than nitrite, and total nitrate/nitrite can be used as an estimate of nitrate.	Same as above.	Optional. Alternative method.

(continued)

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Total Nitrogen	E440.1 (laboratory)	Nutrient needed for microbial growth, may be needed as a substrate amendment.	May indicate need for nitrogen amendment.	Optional.
Total Kjeldahl Nitrogen	E351.1 Colorimetric, Automated Phenate or SM4500-NorgB - Macro-Kjeldahl Method (laboratory)	TKN is the sum of ammonia and organic nitrogen. Nitrogen is an essential nutrient for microbial growth.	Ammonia and organic nitrogen are relatively immobile in soil and groundwater and are susceptible to denitrification under aerobic conditions. A decrease in TKN concentrations may indicate a depletion of nitrogen source and the need for nitrogen amendments.	Optional
Ammonia (NH_4) as Nitrogen	E350.1 Colorimetric, Automated Phenate (laboratory) or Hach method 8155 (field)	Indicator of the biodegradation of organic material. Needed to calculate organic nitrogen concentrations from TKN analyses.	Ammonia and organic nitrogen are relatively immobile in soils and groundwater and are susceptible to denitrification under aerobic conditions. A decrease in ammonia concentrations may indicate a depletion of nitrogen source and the need for nitrogen amendments.	Optional
Nitrite	Hach Method 8155 (field)	Use in combination with Nitrate/Nitrite as nitrogen analysis by Method E353.2 to quantify nitrate and nitrite.	Nitrate levels less than 1.0 mg/L are desirable for anaerobic dechlorination of CAHs.	Optional
Sulfide (H_2S)	E376.2 Colorimetric, Methylene Blue (laboratory) Hach Method 8131 or similar (field)	Byproduct of sulfate reduction. Sulfide typically precipitates with iron minerals, but elevated levels of sulfide may be toxic to dechlorinating microorganisms.	Elevated levels of sulfide in conjunction with elevated levels of CAHs may indicate that iron-compounds should be added to precipitate sulfides and reduce toxicity effects.	Optional. Recommended when elevated levels of sulfate (> 20 mg/L) are present.
Temperature	Direct Reading Meter, E170.1 (field)	General water quality parameter used as a well purging stabilization indicator. Microbial activity is slower at lower temperatures.	Indicator parameter only. Typically used as a well purge stabilization parameter. Microbial activity may be correlated to temperature changes over time.	Measure while purging for each sampling event.

(continued)

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Specific Conductance	E120.1/SW9050, direct reading meter (laboratory or field)	General water quality parameter used as a well purging stabilization indicator. Proportional to dissolved ions present in solution and can provide an approximation of total dissolved solids concentration. May correlate with and support interpretations of other geochemical analyses.	Typically used as a well purge stabilization parameter. Also used to correlate groundwater across an aquifer. If the specific conductance of upgradient and downgradient wells are markedly different, if is likely that they do not sample the same flow path in the aquifer.	Recommended as a well purging parameter.
Major Cations (Fe, Mn, As, Ca, Mg, Na, K)	SW6010B (laboratory)	Some metals may be more mobile under highly reducing conditions.	May be required for regulatory compliance of secondary water quality.	Optional.
Phosphate	E365.1 Semi-Automated Colorimetry (laboratory)	Nutrient needed for microbial growth, may be needed as a substrate amendment.	May indicate need for phosphate amendment.	Optional.
Chloride	E300.0 or SW9050 Ion Chromatography (laboratory), or Hach Chloride test kit model 8-P (field)	General water quality parameter. Chloride is produced by anaerobic dechlorination. Elevated levels of chloride may indicate that dechlorination is occurring if observed concentrations are greater than three times background and consistent with CAH molar concentrations.	Indicator parameter only.	Optional.
Bromide or Iodide	E300.0 or SW9050 - Ion Chromatography (laboratory) or field meter (field)	Used as a conservative groundwater tracer.	Indicator parameter for tracer tests only.	Only used with tracer testing.
Chemical Oxygen Demand	EPA Method 410.4 or 410.1 (laboratory)	A measure of the oxygen required to oxidize all compounds, both organic and inorganic, in water. Used to determine material load in groundwater subject to oxidation.	Indicator parameter only. May be used as an indication of substrate electron acceptor demand. Redundant with TOC or DOC analyses.	Optional.
Biological Oxygen Demand	EPA Method 415.1 (laboratory)	An indirect measure of the concentration of biologically degradable material present in organic wastes.	Indicator parameter only. May be used as an indication of electron acceptor demand. Redundant with TOC or DOC analyses.	Optional.

(continued)

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (continued)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Total Dissolved Solids (TDS)	E160.3	General water quality parameter.	Indicator parameter only, used as an indication of secondary water quality.	Optional. Specific conductance may be adequate for approximating TDS.
Hardness	E130.2 or Field Test Kit	General water quality parameter that is the sum of multivalent metallic cations in solution and for all practical purposes can be represented by the sum of the calcium and magnesium ions.	Indicator parameter only, used as an indication of secondary water quality.	Optional. Redundant analysis if major cations are analyzed (SW6010B).
Volatile Fatty Acids (VFAs) or Metabolic Acids	Laboratory specialty method - Microseeps AM21 G or AM23G	VFAs are an indicator of substrate distribution and are also degradation products of more complex substrates (*e.g.*, carbohydrates or vegetable oils). Fermentation of VFAs produces molecular hydrogen for anaerobic dechlorination.	Measurable concentrations of VFAs (greater than 10 to 20 mg/L) are desirable in the treatment zone. The presence of mg/L concentrations of propionate or butyrate is considered favorable. A lack of measurable VFAs in conjunction with elevated levels of VOCs and alternate electron acceptors indicates additional substrate may be required to sustain an anaerobic treatment zone.	Optional. Useful as a diagnostic tool. Note that VFA analyses are subject to matrix interference in biowall or bioreactor leachate high in organic carbon.
Dissolved Hydrogen	Laboratory specialty method - Microseeps AM20GAX.	Specialized analysis used to determine TEAPs. Hydrogen is the primary electron donor used in anaerobic dechlorination. Hydrogen concentrations between 2 and 11 nanomoles per liter (nM/L) are optimal for efficient reductive dechlorination to occur.	Hydrogen levels less than 2 nM/L in conjunction with elevated levels of VOCs and the absence of TOC indicates additional substrate may be required to promote anaerobic dechlorination.	Optional. May be used as a diagnostic tool after substrate addition.
Phospholipid Fatty Acids	Laboratory specialty method	Indicator of biomass and general composition of the microbial population. Can determine relative levels of microbial stress or starvation.	May be useful to evaluate whether significant changes in microbial populations have occurred, but results do not directly support pass/fail determinations or design changes.	Optional. Only recommended as a diagnostic tool.

(continued)

Table 6.3
Groundwater Analytical Protocol for Permeable Mulch Biowalls and Bioreactors (concluded)

Analysis	Method/Reference (laboratory/field)	Data Use	Performance Expectation	Recommended Frequency of Analysis
Stable Isotope Fractionation	Laboratory specific	The carbon stable isotope compositions are used to determine the extent of biodegradation or quantitatively distinguish between microbial degradation pathways or between biotic and abiotic degradation pathways.	Confirm degradation pathways targeted. May be useful to differentiate between biotic and abiotic degradation processes.	Optional. Only recommended as a diagnostic tool.
DNA sequencing of *Dehalococcoides* species	Laboratory specialty method	Detection of genetic sequences unique to targeted microbial genus and species. See Sections 3 and 6.3.5 for further descriptions of data use.	Positive identification of *Dehalococcoides*-related species indicates potential for complete dechlorination of chlorinated ethenes.	Optional. Only recommended as a diagnostic tool.

NOTES:

Analyses other than those listed in this table may be required for regulatory compliance.

1. "Hach" refers to the Hach Company catalog, 2006.
2. "A" refers to *Standard Methods for the Examination of Water and Wastewater*, 18th edition, 1992.
3. "E" refers to *Methods for Chemical Analysis of Water and Wastes*, USEPA, 1983.
4. "SW" refers to the *Test Methods for Evaluating Solid Waste, Physical, and Chemical Methods*, SW-846, USEPA, 3rd edition, 1986.
5. "ASTM" refers to the American Society for Testing and Materials.

Table 6.4
Analytical Methods and Requirements for Containers, Preservation, Volumes, and Holding Times

Name	Matrix	Analytical Methods	Container	Preservation [a/]	Minimum Sample Volume or Weight	Maximum Holding Time
Soil and Backfill Material						
Volatile Organic Compounds (VOCs)	Soil and Mulch	SW8260B	Encore™ G, Teflon® septum	4°C	3 Encore® 1 x 4 oz jar	48 hours until extraction 14 days (after extraction)
Total Organic Carbon (TOC)	Soil	SW9060 modified	G, Teflon® septum	4°C	1 x 4 oz jar	28 days
Total Iron/Manganese	Soil	SW6010B	G, Teflon® septum	4°C	1 x 4 oz jar	180 days
Acid Volatile Sulfide (AVS)	Soil	Microseeps SOP WC43/WC03 [a/]	G, Teflon® septum	< 0°C	2 x 40 ml	60 days
Chromium Extractable Sulfide (CES)	Soil	Microseeps SOP WC43/WC03	G, Teflon® septum	< 0°C	2 x 40 ml	60 days
Bioavailable Ferric Iron and Manganese	Soil	New Horizons SOP	G, Teflon® septum	4°C	1 x 4 oz jar	60 days
Strong Acid Soluble Ferrous Iron, Ferric Iron and Divalent Manganese	Soil	Microseeps SOP WC43/WC20	G, Teflon® septum	< 0°C	2 x 40 ml	60 days
Percent Solids	Soil	E160.3	G, Teflon® septum	4°C	1 x 4 oz jar	28 days
Total Iron, Manganese, Phosphorous, and Potassium	Much and Sand or Gravel	SW6010B	G, Teflon® septum	4°C	1 x 4 oz jar	180 days
Total Nitrogen	Mulch	E351.3 modified	G, Teflon® septum	4°C	1 x 4 oz jar	28 days
Total Organic Carbon (TOC)	Mulch	SW9060 modified	G, Teflon® septum	4°C	1 x 4 oz jar	28 days
Percent Solids	Mulch	E160.3	G, Teflon® septum	4°C	1 x 4 oz jar	28 days

(continued)

Table 6.4
Analytical Methods and Requirements for Containers, Preservation, Volumes, and Holding Times (continued)

Name	Matrix	Analytical Methods	Container	Preservation [a/]	Minimum Sample Volume or Weight	Maximum Holding Time
Groundwater						
Chlorinated Aliphatic Hydrocarbons (CAHs)	Water	SW8260B	G, Teflon® septum	4°C, HCl to pH < 2	3 x 40 ml	14 days
Perchlorate	Water	E314.1	P	Sterile filtered, 4°C	500 ml	14 days
Explosives	Water	SW8330	G, Teflon® septum	4°C	2 x 40 ml	14 days
Total or Dissolved Organic Carbon (TOC/DOC)	Water	SW9060M	P	4°C, H_2SO_4 to pH < 2	250 ml	28 days
Manganese	Water	Hach® Field Analysis	Provided with Hach Kit	None	100 ml	at well head [a/]
Ferrous Iron	Water	Hach® Field Analysis	Provided with Hach Kit	None	100 ml	at well head [a/]
Major Anions (Cl^-, Br^-, SO_4^-)	Water	E300.0 or SW846 9056	P	4°C	500 ml	28 days
Methane/ Ethane/ Ethene	Water	Kampbell *et al*., 1989 or Microseeps AM20GAX	G, Teflon® septum	4°C	2 x 40 ml	14 days
Dissolved Hydrogen	Water	Microseeps AM20GAX	G, Teflon® septum	4°C	1 x 20 ml	7 days
Alkalinity	Water	Hach® Field Analysis	Provided with Hach Kit	None	100 ml	14 days [a/]
Carbon Dioxide	Water	Hach® Field Analysis	Provided with Hach Kit	None	100 ml	at well head [a/]
Total Inorganic Carbon (TIC)	Water	Microseeps AM20GAX Reported in mg/L as $CaCO_3$	G, Teflon® septum	4°C	2 x 40 ml	14 days
Nitrate and Nitrite as Nitrogen	Water	E353.2	P,G	4°C, H_2SO_4 to pH < 2	500 ml	28 days
Sulfide	Water	Hach® Field Analysis	Provided with Hach Kit	None	100 ml	at well head [a/]
Sulfide	Water	E376.2	P,G	NaOH/ZnOAc to pH>9	100 ml	7 days

(continued)

Table 6.4
Analytical Methods and Requirements for Containers, Preservation, Volumes, and Holding Times (concluded)

Name	Matrix	Analytical Methods	Container	Preservation [a/]	Minimum Sample Volume or Weight	Maximum Holding Time
Major Cations (Fe, Mn, As, Ca, Mg, Na, K)	Water	SW6010B	P,G	4°C, HNO_3 to pH < 2	500 ml	180 days
Volatile Fatty Acids	Water	Laboratory Specific SOP	G, Teflon® septum	4°C	2 x 40 ml	7 days
Phospholipid Fatty Acids	Water	Laboratory Specific SOP	P	4°C	2 x 1 liter	7 days

Acronyms:

P - Polyethylene
G - Glass
H_2SO_4 – Sulfuric acid
HCl - Hydrochloric acid
HNO_3 – Nitric Acid
ZnOAc – Zinc Acetate
NaOH – Sodium Hydroxide

[a/] Note: Hach field analysis should be completed as soon as possible after collection of the sample. The holding times indicated are the maximum times the samples may be held before analysis and still be considered valid. "At Well Head" indicates analysis should be performed immediately following sample collection.

6.5.1 Soil Analyses

Soil analyses are conducted to evaluate the concentrations of contaminants (*e.g.*, CAHs, perchlorate, or explosive compounds) in soil that are a residual source for groundwater contaminant plumes (**Table 6.2**). Often the site is well characterized before an enhanced bioremediation remedy is selected and soil analyses of the contaminants of concern may not be necessary. Analyses of excavated soil may be required for disposal purposes (**Section 5.1**).

Soil samples are also collected to characterize the physical and chemical properties of the aquifer matrix. For example, the f_{oc} in the aquifer matrix is used to calculate retardation factors for dissolved contaminant transport and to estimate the amount of contaminant mass sorbed to the aquifer matrix. Other soil analyses may be used to determine the potential for competing electron acceptor demand or for stimulating abiotic degradation processes. For example, bioavailable ferric iron and manganese may indicate a substantial electron acceptor demand through iron and manganese reduction. Alternatively, a high ferric iron content may be beneficial as a source of iron in the formation of reactive iron sulfides for biogeochemical transformation of CAHs (**Appendix B**).

6.5.2 Biowall Material Analyses

Analysis of biowall materials may be conducted for design or regulatory purposes (pre-installation), or for performance monitoring (post-installation). Differing analyses may be used on either the organic fraction (mulch and compost) or the sand and gravel fraction. Analysis of biowall materials may be conducted for the following reasons:

- ***Potential for Cross Contamination.*** The organic fraction may be analyzed for pesticides or herbicides to satisfy regulatory concerns for cross contamination. Analysis of the sand or gravel fraction is not typically required, but may be considered if the potential for mobilization of heavy metals is an issue.

- ***Nutrient Supply.*** The organic fraction may be analyzed for essential nutrients for microbial growth, including nitrogen, phosphorous, and potassium. A mulch supply low in essential nutrients may be amended with compost, cotton gin trash, or cottonseed meal. Composted mixtures of yard wastes or manures are also viable amendments to supplement the nutrient content of the final mulch mixture.

- ***Fiber Analyses.*** Fiber analyses may be conducted to evaluate the relative percentages of cellulose, hemicellulose, and lignin, as well as ash content. A mulch or compost high in cellulose and low in lignin will provide a source of more readily degradable organic carbon. Procedures used by the NFTA (*e.g.*, Undersander *et al.*, 1993) or by the ASA for analysis of animal forage are suitable for this purpose, and services may be obtained from agricultural laboratories. The NFTA maintains a list of certified laboratories at www.foragetesting.org.

- ***Potential for Stimulating Biogeochemical Transformation.*** The sand and gravel fraction may be analyzed for total or bioavailable iron to determine if it is a suitable or sufficient source of iron for formation of iron monosulfides. Analysis of biowall materials *after* biowall installation (perhaps at 6 to 12 months) for the presence of sulfides (AVS and CES) may be conducted to estimate the amount and mineral state of the sulfide minerals

present (*e.g.*, FeS, see **Appendix B**). These analyses are now available commercially from Microseeps, Inc. (www.microseeps.com). Other methods are described in Kennedy *et al*. (1999) and AFCEE (2002b). The AFCEE document also includes references to other methods described in the literature.

It may also be beneficial to conduct batch tests or column studies using potential biowall materials to determine optimal mixtures (**Section 6.2.2**) prior to design. Simple batch tests may be conducted using potential biowall materials and site groundwater to determine electron acceptor demand (*e.g.*, iron and manganese) and buffering capacity (*e.g.*, pH and alkalinity).

6.5.3 Groundwater Laboratory Analyses

Groundwater sampling and analysis is conducted for evaluation of system performance and for O&M of the biowall or bioreactor system. Analysis of groundwater should include well locations upgradient of the biowall or bioreactor to establish background conditions, and both within and downgradient of the biowall or bioreactor to evaluate changes in contaminant concentrations and biogeochemistry. Groundwater monitoring may also be warranted beneath a biowall or bioreactor to evaluate contaminant bypass and/or the vertical extent of groundwater treatment. Groundwater analysis may be conducted for the following reasons:

- ***Reductions in Contaminant Concentrations.*** Reductions in contaminant concentrations are the primary performance metric for a biowall or bioreactor application.

- ***Groundwater Redox State.*** Anaerobic degradation processes require appropriate reducing conditions. Groundwater ORP and relative changes in concentrations of native electron acceptors or metabolic byproducts may be used to determine the redox state and the predominant terminal-electron accepting processes (TEAPs) that are occurring.

- ***Competing Electron Acceptor Demand.*** In addition to determining the TEAPS that are occurring, measurement of electron acceptors may indicate that a significant amount of substrate is being used to meet native electron acceptor demand. Some anaerobic degradation processes may not be energetically favorable unless a competing electron acceptor is depleted (*e.g.,* inhibition of perchlorate reduction in the presence of DO or nitrate).

- ***Soluble Substrate Supply***. Soluble organic carbon is often measured as an indication of the substrate available for biological processes. TOC, volatile fatty acids (VFAs), or humic and fulvic acids may be analyzed to determine the concentration and form of soluble organic carbon in groundwater. TOC (unfiltered samples) or dissolved organic carbon (DOC, filtered samples) is usually the most cost effective measurement. DOC measurements are usually preferred to screen out interference from particulate matter. In general, levels of TOC or DOC greater than 5 to 10 mg/L are necessary to sustain anaerobic degradation processes.

- ***Secondary Water Quality.*** Analysis of secondary water quality parameters may be required if the aquifer is a potable drinking water supply. In many cases it may be sufficient to document that secondary water quality is not adversely impacted at an appropriate location downgradient of the treatment area. Secondary water quality parameters may include iron, manganese, chloride, TDS, COD/BOD, sulfide, and pH (**Table 2.2**).

- ***Diagnostic Tools.*** Molecular screening techniques and isotope fractionation are two analytical tools that may be used as diagnostic tools when performance is not clear from more conventional groundwater analyses.

Many groundwater analyses are only performed by specialized laboratories (*e.g.*, mineral speciation for iron sulfides, humic and fulvic acids, isotope fractionation, and molecular screening). These analyses are based on standard operating procedures (SOPs) specific to the analytical laboratory. The methods listed in **Table 6.3** and **Table 6.4** are only intended to be guidelines; each site or application may have unique requirements for determining biowall or bioreactor performance.

6.6 GROUNDWATER FIELD ANALYSES

Many of the groundwater chemical parameters listed in **Table 6.3** are measured onsite by field personnel. Some of the measurements are made with direct-reading meters (*e.g.*, YSI Model 650), while others are made using a portable colorimeter (*e.g.*, Hach® Company) or titration kits in accordance with manufacturer-specified procedures. Samples should be collected after stable purging conditions have been obtained, and analysis results should be recorded on a groundwater sampling form. If concentrations of an analyte are greater than the range detectable by a titrimetric or colorimetric method, the analysis should be repeated by diluting the groundwater sample with distilled water until the analyte concentration falls to a level within the range of the method. Common field analyses include the following:

- ***pH, Temperature, and Specific Conductance.*** Because the pH, temperature, and specific conductance of a groundwater sample can change significantly within a short time following sample acquisition, these parameters are measured in the field in a flow-through cell during the purging process. A pH near neutral (6.5 to 7.5) is desirable for most biodegradation processes

- ***Oxidation/Reduction Potential.*** The ORP of groundwater is an indicator of the relative tendency of a solution to accept or transfer electrons. Redox reactions in groundwater are usually biologically mediated; therefore, the ORP of a groundwater system reflects the prevailing TEAPs that are occurring. The ORP of a groundwater sample can change significantly within a short time following sample acquisition and exposure to atmospheric oxygen, therefore this parameter is measured in a flow-through cell during purging. Note that field meter readings for ORP are typically measured against a silver/silver chloride (Ag/AgCl) reference electrode, and should be less than -200 mV for optimal rates of anaerobic dechlorination of CAHs to occur. Redox potentials for reactions listed in the literature (*e.g.*, Thauer *et al.*, 1977 and Bouwer, 1992) involving common groundwater electron acceptors are usually reported as Eh, which is defined as a voltage reading against a SHE. In this case ORP should be less than 0 mV Eh for anaerobic dechlorination of CAHs to occur (see **Section B.3** in **Appendix B** for further discussion). ORP readings higher than these levels, in conjunction with elevated levels of DO and the absence of TOC/DOC, may indicate that additional substrate is required to promote anaerobic dechlorination of CAHs. Less reducing conditions may be required for degradation of perchlorate or explosive compounds.

- ***Dissolved Oxygen.*** DO measurements are typically made with a sensor in a flow-through cell, or a downhole oxygen sensor. Multiple measurements should be taken during well purging until stabilization criteria are met, prior to sample acquisition. The final

measurement of DO made at the completion of the well purge is typically reported as the final stabilized reading.

- ***Manganese.*** Concentrations of manganese concentrations may be measured in the laboratory or in the field. Colorimetric analysis with a portable colorimeter such as the Hach® Model DR/820. USEPA-approved Hach® Method 8034 (range of 0.1 to 20.0 mg/L) is commonly used to prepare and analyze samples for soluble manganese. Elevated concentrations of manganese relative to background is an indication that manganese reduction is occurring.

- ***Ferrous Iron.*** Concentrations of ferrous iron may be measured in the field via colorimetric analysis with a Hach® DR/820 portable colorimeter. USEPA-approved Hach® Method 8146 is commonly used to prepare and analyze samples for ferrous iron (range of 0.01 to 3.0 mg/L). Elevated concentrations of ferrous iron relative to background is an indication that iron reduction is occurring.

- ***Sulfate and Hydrogen Sulfide.*** Sulfate and hydrogen sulfide may be measured in the laboratory, or in the field via colorimetric analysis with a Hach® DR/820 portable colorimeter. Samples for laboratory analysis must be preserved (**Table 6.4**). USEPA-approved Hach® Method 8051 (0.1 to 70 mg/L sulfate) and Method 8131 (0.01 to 0.60 mg/L hydrogen sulfide) are typically be used to prepare and analyze the samples for sulfate and hydrogen sulfide, respectively. Sulfide determinations in the field that are out of range and require dilution should be diluted with water that is free of oxygen. Otherwise, the oxygen in the dilution water will oxidize a major portion of the sulfide before it can be measured. This dilution water can be prepared by boiling distilled water, then purging with oxygen free nitrogen as it cools, or by simply purging with oxygen free nitrogen.

- ***Carbon Dioxide.*** Carbon dioxide is a byproduct of biological reactions and can be used to evaluate biological activity in the groundwater system. Carbon dioxide may be measured in the field via titrimetric analysis using USEPA-approved Hach® Method 8205 (1.0 to 1,000 mg/L), or equivalent

- ***Alkalinity.*** Alkalinity in groundwater helps buffer the groundwater system against acids generated through both aerobic and anaerobic biodegradation processes. Alkalinity may be measured in the field via titrimetric analysis using USEPA-approved Hach® Method 8203 (0.1 to 40 mg/L or 40 to 400 mg/L, as calcium carbonate).

Other analytes that may be measured in the field include nitrate, nitrite, ammonia, hardness, chloride, or turbidity. The user should consult the manufacturer's instructions for the appropriate test methods. In addition, many of the parameters listed in this section are subject to interference from other compounds or ions that may be elevated under the highly reducing conditions present in a biowall or bioreactor. Confirmation of field results with laboratory analyses may be performed if field results are inconsistent or difficult to discern.

6.7 FIELD QA/QC PROCEDURES

Field QA/QC procedures include collection of field duplicate and MS/MSD samples; decontamination of all non-dedicated equipment that contacts the sample medium before and after each use; use of analyte-appropriate containers; and use of chain-of-custody procedures for

sample handling and tracking. Samples transferred to the laboratory for analysis should be clearly labeled to indicate sample number, location, matrix (*e.g.,* groundwater), and analyses requested. Samples should be preserved in accordance with the analytical methods to be used (**Table 6.4**).

Two or more spare samples should be collected concurrently, and by the same method as, the primary sample. The spare samples can be used to replace a sample that is lost during analysis, for a laboratory duplicate, or for a matrix spike sample. The primary sample and all of the spare samples should not be trusted to the same analytical batch or run on an automated instrument. MS/MSDs are prepared in the laboratory and used to establish matrix effects for samples analyzed for the contaminants of concern. Sufficient extra sample volume should be submitted to the laboratory to allow matrix spike preparation and analysis. A typical field QA/QC sampling program is summarized in **Table 6.5**.

Table 6.5
Typical QA/QC Sampling Program

QA/QC Sample Type	Minimum Frequency to be Collected and Analyzed	Analytes
Duplicates	10 percent of groundwater and soil samples	VOCs; perchlorate; explosives; methane, ethane, and ethene; TOC/DOC; dissolved inorganics; field test kit analyses
Trip Blanks	One per sample shipment containing VOCs	VOCs only
Matrix Spike and Matrix Spike Duplicate Samples	5 to 10 percent of groundwater and soil samples	VOCs, perchlorate, explosives only

Notes: QA/QC – Quality Assurance/Quality Control; VOC – volatile organic compound, TOC – total organic carbon; DOC – dissolved organic carbon.

In order to provide complete documentation of the sampling event, detailed records should be maintained by the field scientist. Groundwater sampling information should be recorded on a groundwater sampling form. Bound field logbooks should be maintained by the field team members to provide a daily record of significant events, observations, and measurements during the field program. All information pertinent to the field survey and/or sampling should be recorded in the logbooks.

The laboratory will typically add any necessary chemical preservatives prior to shipping the sample containers to the field. Samples will be prepared for transportation to the analytical laboratory by placing the samples in a cooler containing ice to maintain a shipping temperature of not more than 4 degrees Celsius (°C). Chain-of-custody forms are completed for each shipment of samples to track their movement. The chain-of-custody form should include sample information (*e.g.*, sample identification, type, date and time of collection, any preservative added in the field), analyses requested, and the signature of each person receiving and relinquishing the samples.

6.8 AQUIFER TESTING

Aquifer testing is conducted to 1) evaluate groundwater hydraulics (rate of flow), 2) measure the relative difference in permeability between the formation and the biowall to ensure that

contaminant bypass does not occur, and 3) evaluate any potential impact of the bioremediation processes on the hydraulic conductivity of the aquifer. Aquifer tests may include single well tests such as slug tests, constant drawdown tests, borehole flow meter surveys, pumping tests using multiple observation wells, or tracer studies.

Slug tests are the easiest to conduct, and are a single-well hydraulic test used to determine the hydraulic conductivity of an aquifer in the immediate vicinity of the screened interval of the tested well (American Society for Testing and Materials [ASTM], 1997). Slug tests can be used for both confined and unconfined aquifers that have a transmissivity of less than 7,000 square feet per day. Slug testing can be performed using a rising head and/or a falling head test. Slug tests should be conducted immediately following installation of the biowall or bioreactor systems to establish baseline conditions, and during scheduled performance monitoring events. Data obtained during slug testing may be analyzed using the method of Bouwer and Rice (1976) and Bouwer (1989) for unconfined or semi-confined conditions.

Tracer studies may also be conducted to evaluate groundwater seepage velocity, dispersivity, and residence time. Tracer tests may be useful in some cases to evaluate flow along preferential flow paths or bypass under or around a biowall trench.

The presence of preferential flow paths or zones of high groundwater flow through a biowall may result in contaminant bypass or zones of incomplete treatment. Borehole flow meter surveys may be used to measure the presence of high flow zones. The use of borehole flow meters is described in USEPA (1998b). Alternately, tracer tests may be conducted to evaluate groundwater hydraulics and the presence of preferential flow paths. These methods are equipment and labor intensive, and are generally reserved for diagnostic purposes.

SECTION 7

DATA INTERPRETATION AND REPORTING

7.1 INTERPRETATION OF CONTAMINANT DATA

Several methods are available to assess the effectiveness of enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls and *in situ* bioreactors. These include evaluations of changing contaminant concentration/mass over time, changes in groundwater geochemistry, and an evaluation of the efficiency (rate) and extent of biodegradation. The following subsections discuss the contaminant and geochemical changes that occur during enhanced anaerobic bioremediation, and some of the common tools and methods used to evaluate and report the effectiveness of a permeable mulch bioremediation system.

Groundwater contaminant and geochemical data collected during system monitoring can be used to demonstrate whether aquifer redox and geochemical conditions have been modified as planned, and to detect changes in environmental conditions that may optimize or reduce the efficacy of the biowall system. Evaluation of field data as it applies to natural attenuation and enhanced bioremediation of chlorinated solvents is described in further detail in USEPA (1998a) and AFCEE *et al.* (2004).

Monitoring parameters that indicate that indicate whether ***geochemical conditions*** are optimal for anaerobic degradation processes to occur include the following:

- DO concentrations are less than 0.5 mg/L and ORP values are less than -200 mV relative to a Ag/AgCl electrode (or less than 0 mV relative to a SHE), indicating that a reducing environment conducive to anaerobic degradation processes has been achieved (Sims *et al*., 1990).
- Production of ferrous iron and a reduction in sulfate levels further indicate that groundwater conditions are sufficiently reducing for anaerobic dechlorination to occur.
- The production of methane, indicating that anaerobic fermentation is occurring.
- Hydrogen concentrations are greater than 1.0 nanomoles per liter (nmol/L), indicating that sufficient primary electron donor is present to sustain anaerobic dechlorination of CAHs.

Monitoring parameters that indicate ***biotic anaerobic reductive dechlorination*** of CAHs may be occurring include the following:

- Concentrations of parent compounds (*e.g.*, PCE, TCE, 1,1,1-TCA, or CT) are reduced.
- Dechlorination products are being produced (*e.g.*, *cis*-1,2-DCE, VC, CA, or chloromethane [CM]).

- Ethene and/or ethane are being produced.

Monitoring parameters that indicate whether ***biogeochemical transformation*** of CAHs may be occurring include the following:

- Concentrations of parent compounds (*e.g.*, PCE, TCE, 1,1,1-TCA, or CT) are reduced.
- Dechlorination products are ***not*** accumulating (*e.g.*, *cis*-1,2-DCE, VC, CA, or CM).
- Sulfate and iron reduction are evident as primary TEAPs.

Contaminant data comprise the primary line of evidence used to demonstrate that anaerobic degradation of CAHs in groundwater is occurring. A reduction in the concentrations of parent compounds coupled with the appearance of dechlorination products can be used to determine the rate and extent to which degradation is occurring.

Similarly, reductions in concentrations of perchlorate and explosive compounds (RDX, TNT) may be used to determine the rate at which these compounds are being degraded. Anaerobic degradation of perchlorate, RDX, and TNT may also produce intermediate degradation products, which may be measured as further evidence that these compounds are being degraded. Intermediates of perchlorate (chlorate and chlorite) are not stable in natural groundwater systems and readily degrade to innocuous chloride. However, like chlorinated solvents, the persistence of intermediates from the anaerobic degradation of RDX or TNT is not favorable because the intermediates are possibly as toxic as the parent compound. Many, but not all, of the possible intermediates of RDX (*e.g.*, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine [MNX], hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine [DNX], or hexahydro-1,3,5-trinitroso-1,3,5-triazine [TNX]) and of TNT (*e.g.*, 2,4- and 2,6-dinitrotoluene [DNT]) can be measured (*e.g.*, see target analyte list for USEPA Method SW-846 8330 modified). Explosive compounds do not have promulgated USEPA National Primary Drinking Water Standards, but many may be found on the USEPA Region 9 Table of Preliminary Remediation Goals (PRGs). Practitioners should review applicable regulations for the presence of regulated intermediate compounds for their site.

Directly assessing biological activity at a field site based on monitoring data can be difficult. Geochemical evaluations are focused on demonstrating that the "footprints" of the expected degradation processes are present. These include indications that alternate electron acceptors have been depleted via utilization of organic substrate. For example, DO, sulfate, and ORP may be reduced, and ferrous iron and manganese may increase. The availability of organic substrate is often measured and tracked by measuring parameters such as TOC or VFAs.

Any evaluation of site data should use multiple converging lines of evidence for system performance evaluation and decision-making. A certain percentage of conflicting data may be observed. For this discussion, conflicting data are defined to be individual or multiple results that do not correlate with expected trends or subsurface geochemical conditions. Conflicting data can arise from systematic errors in sampling or analysis. For example, a high DO reading (*e.g.*, 3.0 to 10 mg/L) in the same well that contains milligram per liter concentrations of methane typically represents a systematic error, since the production and persistence of methane is inconsistent with the presence of oxygen. The presence of conflicting data should initiate a quality assurance exercise to detect and minimize any systematic errors. But major system

modifications should not be initiated due to the presence of conflicting data if multiple lines of evidence support acceptable system performance.

Evaluation of monitoring data should include assessment of whether contaminant mass loss may be due to anaerobic degradation processes or due to non-destructive processes such as sorption, dilution, or dispersion (*e.g.*, see USEPA, 1998a). Upgradient wells and wells with historical data trends may be used to account for the effects of natural attenuation over time. Monitoring results may be used to evaluate the following:

- Demonstrate the efficacy of enhanced anaerobic degradation at a particular site by providing site-specific field data regarding contaminant reduction; and
- Determine whether appropriate reducing conditions are achieved and sustained, or the need to replenish the biowall or bioreactor using secondary substrates.

A laboratory column study or a field demonstration may also be implemented to help define design parameters for full-scale applications based on mulch substrate requirements, degradation reaction rates, and the extent of the reactive zone required to meet remedial objectives. Examples of laboratory column studies include a study described by Shen and Wilson (2007) for chlorinated solvents, a study described by Perlmutter *et al.* (2001) for perchlorate, and a study described by GSI (2005) and Ahmad *et al.* (2007a) for RDX. Examples of biowall demonstrations are available on the AFCEE Tech Transfer web site (http://www.afcee.brooks.af.mil/products/techtrans/bioremediation/BIOREMresources.asp), including work plans for biowalls at Altus AFB, Oklahoma; F.E. Warren AFB, Wyoming; and Ellsworth AFB, South Dakota. Additional work plans and reports are posted on AFCEE web site as demonstration projects are completed. Once a bench-scale or pilot-scale test has defined critical design criteria, full-scale design can be completed with greater confidence.

7.1.1 Changes in Contaminant Concentrations

Evaluating the effectiveness of a permeable mulch biowall or bioreactor includes an assessment of reductions in contaminant concentration or mass. Reductions in post-installation contaminant concentrations relative to pre-installation baseline conditions or to concentrations upgradient of the biowall can be used to show that the process is working to destroy contaminant mass. It is important that the temporal and spatial data demonstrate a clear and meaningful trend in contaminant concentration or mass over time at appropriate monitoring locations

For CAHs, the evaluation also includes changing molar concentrations of parent and dechlorination products over time. A change in the molar ratio of parent compounds to dechlorination products can be useful in evaluating the extent to which dechlorination is occurring, or the extent to which degradation may be attributed to abiotic reactions such as biogeochemical transformation.

A uniform decrease in total molar concentration may not always be observed downgradient of the biowall. This is not atypical for enhanced anaerobic bioremediation remedies in the early stages of operation. As concentrations of TCE in groundwater are lowered, TCE sorbed to the aquifer matrix will desorb due to equilibrium partitioning. In addition, TCE in groundwater within low permeability sediments will slowly diffuse back into more permeable zones where groundwater flow primarily occurs.

Enhanced desorption of TCE has also been observed to occur in the presence of elevated levels of soluble organic carbon in groundwater (Payne *et al.,* 2001). Due to the anaerobic conditions produced, this often results in the dechlorination of desorbed TCE to *cis*-1,2-DCE and VC. Because *cis*-1,2-DCE and VC do not sorb as strongly as TCE, the result is an increase in the apparent molar concentration of *cis*-1,2-DCE and VC in groundwater due to mass transfer of TCE from the sorbed phase. This effect often masks (underestimates) the amount of biodegradation that is observed from aqueous phase concentrations alone. Because contaminant mass is being removed from the aquifer system, a reduction in total molar concentrations downgradient of the biowall sections should ultimately (albeit gradually) be observed over time.

The presence of intermediate degradation products within or downgradient of a biowall or bioreactor reaction zone provides evidence that sorption is not as prominent a removal process compared to degradation. Mulch materials have been sampled for CAHs to assess the extent of sorption at the OU-1 and SS-17 biowalls at Altus AFB, Oklahoma and at the B301 biowall at Offutt AFB, Nebraska. **Table 7.1** summarizes sampling results from the three sites.

Table 7.1
Summary of Chlorinated Ethenes in Groundwater and Biowall Materials

TCE in Groundwater		Biowall Materials				
Upgradient (µg/L)	**In Biowall (µg/L)**	**Number of Samples**	**TOC (mg/kg)**	**TCE (µg/kg)**	***cis*-1,2-DCE (µg/kg)**	**VC (µg/kg)**
OU-1 Biowall Samples, Altus AFB, Oklahoma (April 2005)						
1500	<12	7	15,000 to 41,000	<3.1 to 25	3.0 to 760	<6.1 to 210
SS-17 Transect B Biowall Samples, Altus AFB, Oklahoma (October 2006)						
335 to 11,000	13.4J to 50.6	2	5,110 to 25,300	<6.7	<6.7	<6.7
B301 Biowall Samples, Offutt AFB, Nebraska (July 2003)						
790	850	3	16,400 to 20,000	<1.0	<1.0	<1.0

NOTES: µg/L = micrograms per liter; mg/kg = milligrams per kilogram; µg/kg = micrograms per kilograms.
Source: Parsons, 2007c; Parsons, 2007d; and GSI, 2004.

Concentrations of TCE, *cis*-1,2-DCE and VC were below detection for samples of biowall material collected from the B Transect of the SS-17 biowall system at Altus AFB, Oklahoma, and from the B301 biowall at Offutt AFB, Nebraska. Concentrations of TCE in groundwater upgradient of these biowalls were several hundred micrograms per liter or higher. Concentrations of TCE in biowall samples collected from the OU-1 biowall at Altus AFB, Oklahoma were also low, ranging from below detection up to 25 micrograms per kilogram (µg/kg).

Concentrations of *cis*-1,2-DCE and VC in biowall samples from OU-1 were elevated in some samples, ranging up to 760 µg/kg and 210 µg/kg, respectively. Concentrations of *cis*-1,2-DCE and VC were also elevated in groundwater within the OU-1 biowall (data listed in **Appendix F.2**). This suggests that chlorinated compounds sorbed to the mulch material will reflect the degradation that is occurring within the biowall. Contaminant mass in the water-filled porosity of the mulch is also measured during the analysis, which may account for some of the elevated levels of *cis*-1,2-DCE and VC observed.

In any event, field data do not suggest that sorption is a significant long-term removal mechanism. Initial reductions in concentrations of CAHs may be due to sorption, but after several months the mass of CAHs sorbed to the biowall material will approach an equilibrium. After this initial acclimation period, reductions in CAHs can be attributed to degradation processes.

There are several ways to present data showing changes in contaminant concentrations and plume configuration over time after system installation. One method consists of preparing isopleth maps of contaminant concentrations over time. The use of vertical cross-section contour plots oriented along the path of groundwater flow is also recommended where multi-level sampling is conducted to understand the vertical distribution of substrate and contaminant mass. Evaluating the change in concentration and the molar ratios of parent compounds to dechlorination products over time or distance can be useful in determining the efficacy of anaerobic degradation stimulated in the biowall or bioreactor system.

7.1.1.1 Calculation of Molar Concentrations of CAHs

Evaluating trends in molar concentrations and ratios for CAHs can often be more informative than evaluating changes in the parent/dechlorination product concentrations alone (*e.g.*, using concentrations in units of µg/L). The molecular weights of the various parent compounds and dechlorination products vary, with the dechlorination products having progressively lower molecular weights (**Table 7.2, Appendix C.1**). As a result, the reductive transformation of a given mass of TCE, for example, does not produce the same mass of DCE (*e.g.*, anaerobic dechlorination of 100 µg/L of TCE would produce 74 µg/L of DCE).

Table 7.2
Molecular Weights for Various Chlorinated Compounds

Compound	Formula	Molecular Weight (grams/mole)
Tetrachloroethene (PCE)	C_2Cl_4	165.83
Trichloroethene (TCE)	C_2HCl_3	131.39
Dichloroethene (DCE)	$C_2H_2Cl_2$	96.95
Vinyl Chloride (VC)	C_2H_3Cl	62.51
Ethene	C_2H_4	28.05
Trichloroethane (TCA)	$C_2H_3Cl_3$	133.41
Dichloroethane (DCA)	$C_2H_4Cl_2$	98.96
Chloroethane (CA)	C_2H_5Cl	64.51
Ethane	C_2H_6	28.05
Tetrachloromethane/ Carbon Tetrachloride (CT)	CCl_4	153.82
Trichloromethane/ Chloroform (CF)	$CHCl_3$	119.38
Dichloromethane (DCM)/ Methylene Chloride (MC)	CH_2Cl_2	84.93
Chloromethane (CM)	CH_3Cl_1	50.49
Methane	CH_4	39.49

Conversion of conventional concentrations (*e.g.*, µg/L) to molar concentrations (moles per liter [mol/L]) facilitates assessment of the degree to which reductive transformations occur, because transformation of 1 mole of TCE yields 1 mole of DCE. This conversion is accomplished by dividing the conventional concentration by the molecular weight of the compound. Decreases in the molar concentration of total chlorinated ethenes, for example, indicate that chlorinated ethene mass is being lost and that transformation of these compounds to non-toxic end products is occurring. The steps required to calculate molar concentrations and ratios to determine trends over time can be found in Section 6 of AFCEE *et al.* (2004).

7.1.1.2 Concentration versus Time or Distance Plots

Plots of concentrations of parent compounds and dechlorination products over time or distance within the reaction zone can be useful in evaluating the effectiveness of enhanced bioremediation using a permeable mulch biowall or bioreactor. **Figure 7.1** shows conceptually how concentrations of individual compounds change over time as ***sequential dechlorination*** proceeds. Starting concentrations of 100 nanomoles (nmol) (16,583 µg/L) of PCE, 50 nmol (6,570 µg/L) of TCE, and 10 nmol (970 µg/L) of DCE are sequentially dechlorinated to ethene at rates ranging from 0.5 per day for PCE to 0.125 per day for VC. Ethene is not degraded (0.0 per day) to show complete molar conversion; in reality, ethene may be further degraded in the field and molar conservation is rarely observed.

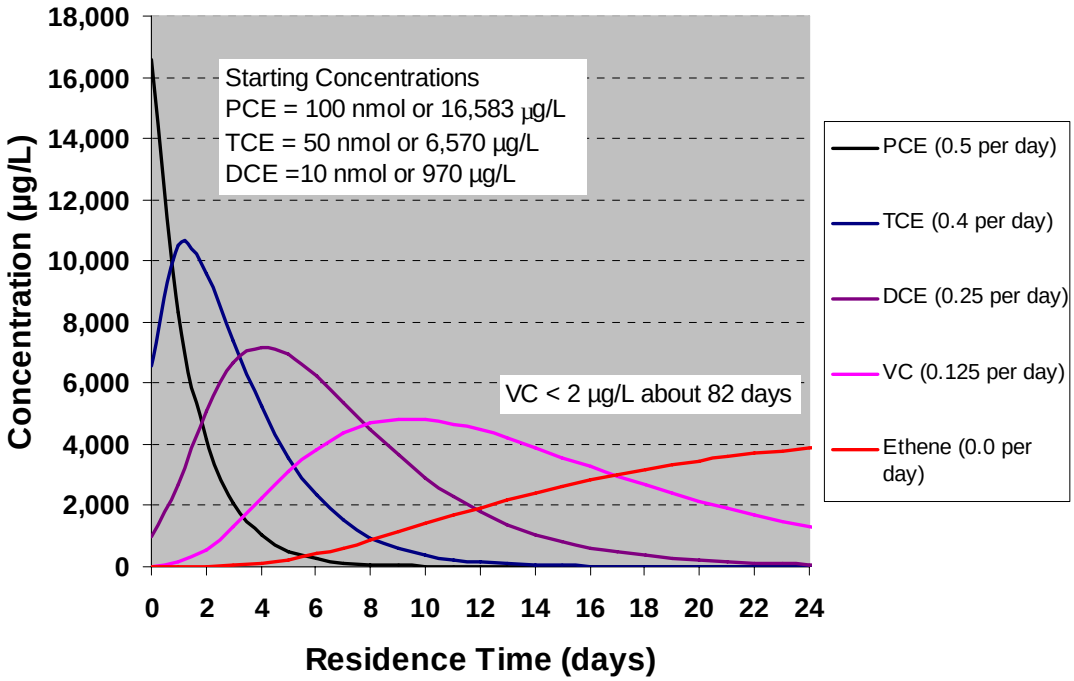

Figure 7.1 Changes in Molar Concentrations of Chloroethenes Over Time With Sequential Dechlorination at Specified Degradation Rates

In this illustration, VC continues to increase until PCE, TCE, and DCE are depleted (around Day 9), and then begins to decrease. The time required for VC to be reduced to less than 2.0 µg/L is approximately 82 days. A residence time of this magnitude is difficult to achieve with a biowall or bioreactor system without recirculation or multiple biowalls.

Conversely, **Figure 7.2** shows a pattern of expected change in contaminant concentrations over distance when attenuation is due to destructive processes that do not produce intermediate dechlorination products, such as ***biogeochemical transformation***. Starting concentrations of 100 nmol (16,583 µg/L) of PCE and 50 nmol (6,570 µg/L) of TCE are degraded at rates of 0.5 per day and 0.4 per day (same rates used in **Figure 7.1**) by an abiotic pathway that does not produce sequential dechlorination products. A starting concentration of 10 nmol (970 µg/L) of DCE is sequentially dechlorinated to VC and ethene at rates identical to **Figure 7.1**. In practice, DCE may also be degraded by abiotic processes that do not produce VC.

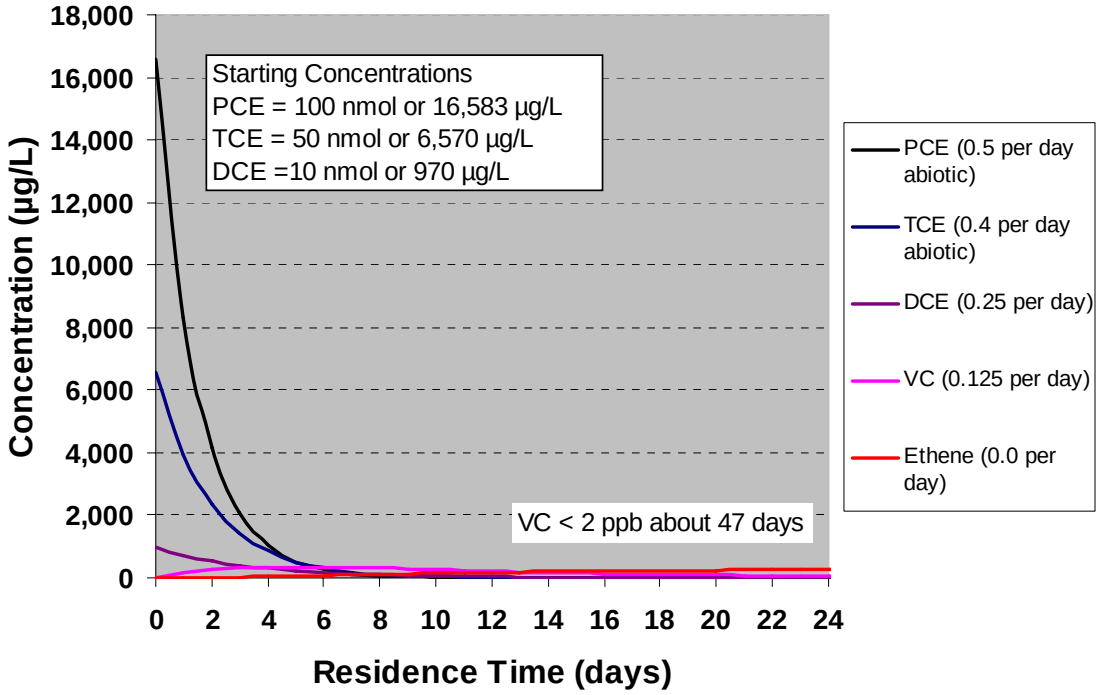

Figure 7.2 Changes in Molar Concentrations of Chloroethenes Over Time With Biogeochemical Transformation at Specified Degradation Rates

Without sequential dechlorination, the concentrations of PCE, TCE, and DCE uniformly decline and the ratios of these compounds remain relatively constant. TCE and DCE are depleted much more rapidly than with sequential dechlorination, even though individual rates of degradation are the same. Even though DCE and VC are conservatively degraded in sequential fashion in this illustration, the time for VC to degrade to less than 2.0 µg/L is 47 days, which is approximately half the residence time as depicted on **Figure 7.1**.

Stimulation of biogeochemical transformation of CAHs may significantly reduce the residence time and size of the reaction zone required for a biowall or bioreactor system. In addition, plotting actual contaminant concentrations over time or distance within the reaction zone may provide insight into which degradation process is dominant. ***In many cases the changes in CAH concentrations will exhibit a mixture of the trends illustrated on Figures 7.1 and 7.2; both processes may be occurring simultaneously.***

Figure 7.3A and **Figure 7.3B** depict real-world data showing how concentrations of individual compounds changed over time during a biowall pilot test at the Ash Landfill, Seneca Army Depot Activity, New York (see **Appendix F.1**). It is clear from these plots that sequential

anaerobic dechlorination occurred with a temporal accumulation of the intermediate dechlorination products *cis*-1,2-DCE and VC at approximately 13 weeks after biowall installation.

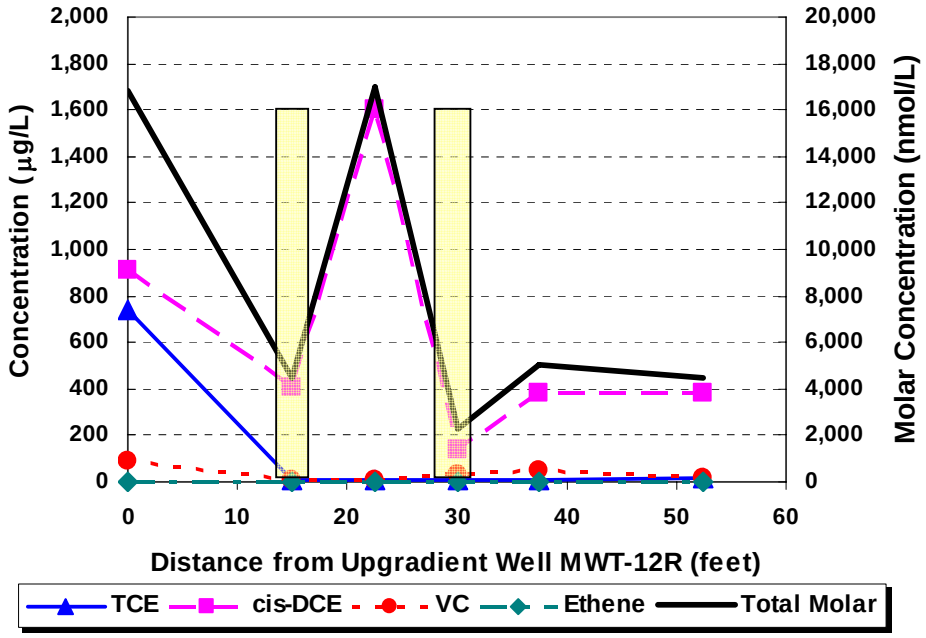

Figure 7.3A Concentrations of Chloroethenes and Total Molar Chloroethenes Along the Northern Flowpath at 13 Weeks

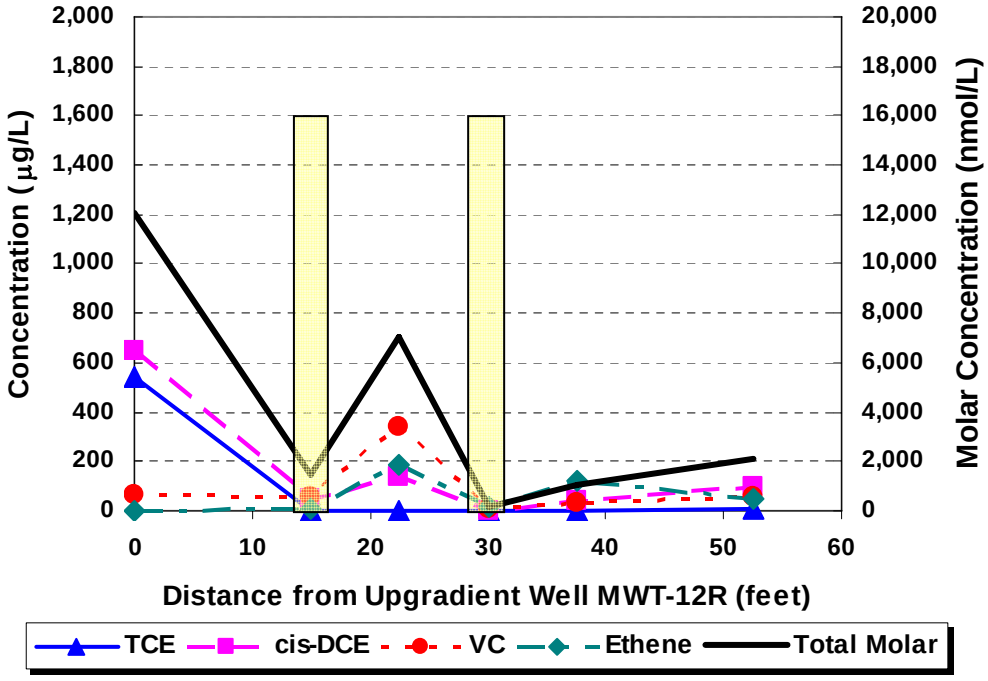

Figure 7.3B Concentrations of Chloroethenes and Total Molar Chloroethenes Along the Northern Flowpath at 27 Weeks

The overall reduction in total molar concentrations of chlorinated ethenes between the upgradient location and the second biowall is apparent at both 13 and 27 weeks post-installation. A large increase in total molar concentration, predominately *cis*-1,2-DCE, was observed at 13 weeks between the first and second biowall, presumably due to enhanced desorption or back-diffusion of TCE. However, by 27 weeks the spike in total molar concentration between the two biowalls is much less pronounced and is predominately comprised of VC. Increases in ethene/ethane are apparent, indicating that the biowall system has acclimated to highly reducing conditions and complete sequential dechlorination is occurring. Once the more highly chlorinated compounds are depleted (*i.e.*, TCE), then concentrations of the less chlorinated compounds (*cis*-1,2-DCE and VC) should continue to decline.

The practitioner should exercise care in interpreting early sampling results that indicate a temporal accumulation of intermediate dechlorination products; this trend may be due to kinetic disparity where the intermediate dechlorination product is being generated faster than it is degraded, and not to an absence of appropriate dechlorinating microorganisms.

Total molar concentrations of chlorinated ethenes within the biowalls at the Ash Landfill site were much lower relative to locations outside of the biowalls. If TCE and *cis*-1,2-DCE were being degraded by sequential reductive dechlorination to VC and ethene/ethane alone, then the total molar concentration would be expected to remain steady across the biowall treatment zone. Because molar conservation of TCE and *cis*-1,2-DCE to VC and ethene/ethane was not observed within the biowalls for this site, it is possible that alternative anaerobic degradation processes (*e.g.*, biogeochemical transformation or anaerobic oxidation) of chlorinated ethenes also may have been occurring.

7.1.1.3 Changes in Total Molar Concentration

Figure 7.4 presents a plot of the molar concentration of total chlorinated ethenes (PCE, TCE, DCE, plus VC) versus distance along a well transect oriented parallel to the direction of groundwater flow for a permeable mulch biowall at Altus AFB, Oklahoma (**Appendix F.3**). Note that ethene and ethane were purposely left out of the calculation because they do not represent contaminant mass (they are innocuous byproducts). The decreasing concentration of total molar chlorinated ethenes within the biowall shown on **Figure 7.4** provides reasonable evidence that contaminant mass is being completely destroyed and converted to innocuous end products.

Figure 7.5 shows molar concentrations of TCE, total DCE, VC and ethene/ethane for the same well transect shown on **Figure 7.4** in November 2003. TCE was reduced to below detection in the biowall well, and DCE was also significantly reduced. However, little VC or ethene/ethane was produced. This degradation signature between the upgradient monitoring location and the biowall is indicative of biogeochemical transformation as illustrated on **Figure 7.2**. It is notable that groundwater at this site is high in sulfate (approximately 2,000 mg/L), and that river sand high in oxidized (ferric) iron was used for backfill. Sampling and analysis of sulfides in the biowall materials (**Appendix F.2** and Kennedy and Everett, 2003) detected elevated levels of sulfide by AVS extraction, indicating the sulfide was present as iron mono-sulfide (FeS). Sufficient amounts of sulfide were measured to account for the observed degradation of TCE (**Appendix D**).

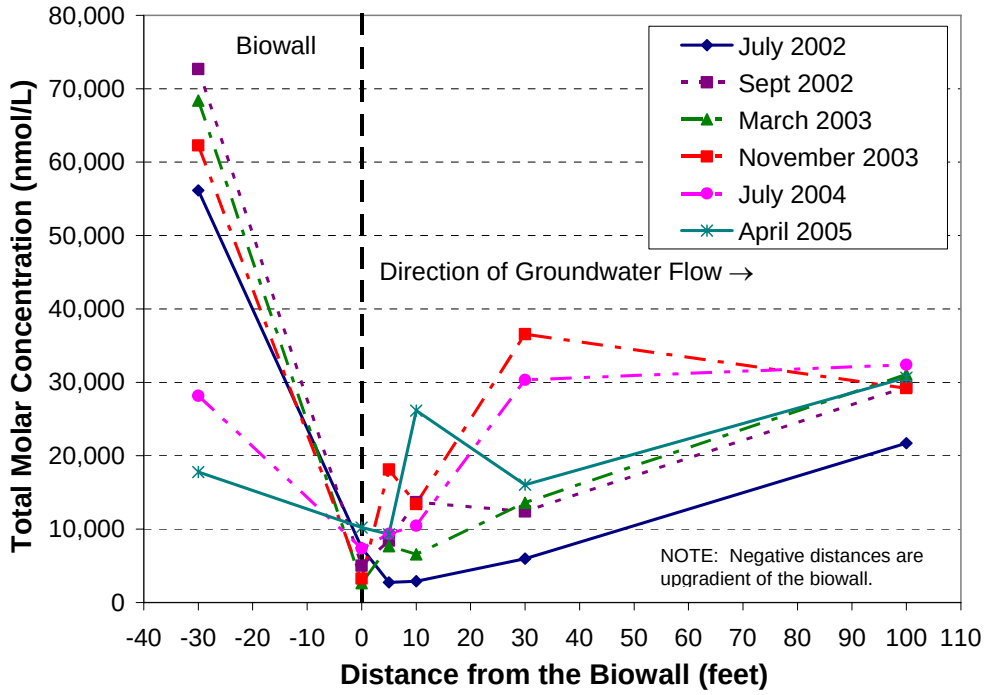

Figure 7.4 Total Molar Concentration over Distance along a Groundwater Flowpath through a Mulch Biowall at Altus AFB, Oklahoma

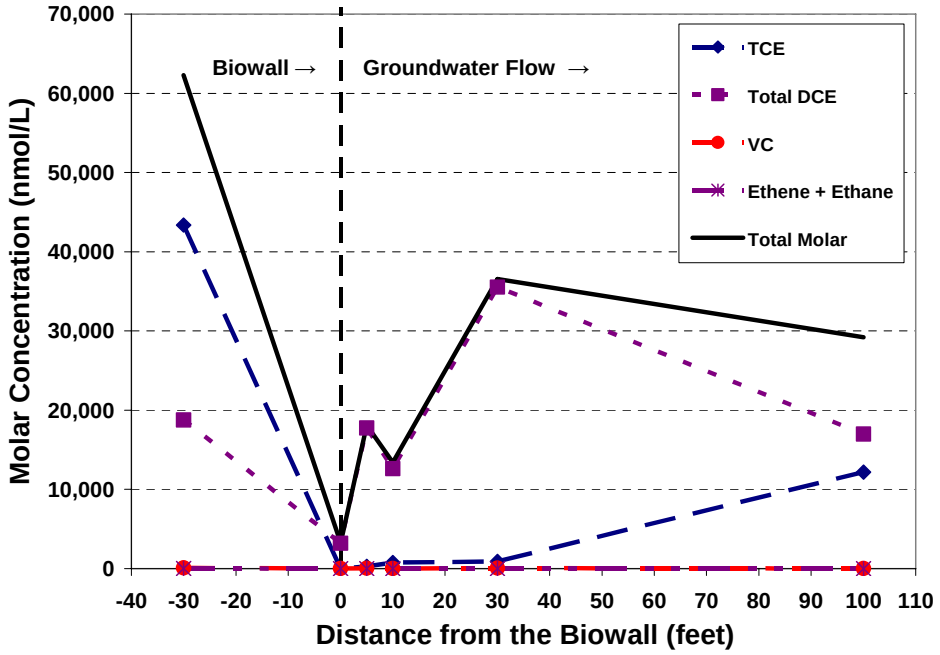

Figure 7.5 Molar Concentrations over Distance in November 2003 along a Groundwater Flowpath through a Mulch Biowall at Altus AFB, Oklahoma

Downgradient of the biowall the concentration of *cis*-1,2-DCE rebounds sharply. Concentrations of sulfate (listed in **Appendix F.2**) downgradient of the biowall also rebound, suggesting that biogeochemical transformation may not be sustained outside of the biowall. Reducing conditions downgradient of the biowall are evident, and the rebound in *cis*-1,2-DCE may be due to desorption or back-diffusion of TCE and subsequent biotic transformation to *cis*-1,2-DCE. Alternatively, the rebound in concentrations may be due to mixing with untreated groundwater; for example, an increase in TCE was observed 100 feet downgradient of the biowall.

7.1.2 Changes in Molar Fractions

A plot of the molar fraction or ratio of CAHs over time is another method used to determine if biodegradation has been stimulated. In particular, this method is often employed when there is a constant or continuing source of contaminant mass entering a treatment system. ***In this case, the total molar concentration may remain elevated or even increase due to a continuing mass influx or desorption, but an increase in the molar ratio of dechlorination products will demonstrate that sequential anaerobic dechlorination is occurring.***

The theoretical change in concentration over time or distance that is expected during sequential reductive dechlorination of chlorinated ethenes is shown on **Figure 7.1**, and may be summarized in the following steps:

1. PCE or TCE is the predominant contaminant source.
2. As PCE and TCE are reduced, DCE levels increase.
3. DCE decreases as PCE and TCE are depleted and as DCE is converted to VC.
4. Finally, VC decreases as DCE is depleted and VC is converted to ethene.

Figure 7.6 is a plot of molar fractions of individual chlorinated ethenes and ethene/ethane for the same monitoring transect shown on **Figure 7.3** for the North Transect at the Ash Landfill pilot test at Seneca Army Depot Activity, New York. Reductive dechlorination has proceeded from TCE and DCE being predominant upgradient of the first biowall, to conversion of TCE to DCE and DCE to VC within the first biowall. Following the path of groundwater flow along the monitoring transect, dechlorination has proceeded to conversion of VC to ethene from the first to the second biowall. Changes in molar fractions clearly indicate that sequential anaerobic dechlorination is occurring.

Within the second biowall, ethene/ethane accounts for over 80 percent of the total molar concentration of chlorinated ethenes and ethene/ethane. Downgradient of the second biowall the relative percentage of DCE and VC rebound to over 20 percent of the total molar concentration. This may be due to several factors, including a residual source of contaminant mass in the sorbed phase, mixing with untreated groundwater, or a less robust reaction zone downgradient of the biowall trench.

For a recirculation bioreactor, it may be more appropriate to look for changes in molar concentration over time at a single monitoring well, perhaps comparing results within or immediately beneath the bioreactor with a downgradient monitoring well as a measure of the overall bioreactor effectiveness. Comparing influent concentrations to concentrations within the interior of the bioreactor can also provide a measure of removal efficiency.

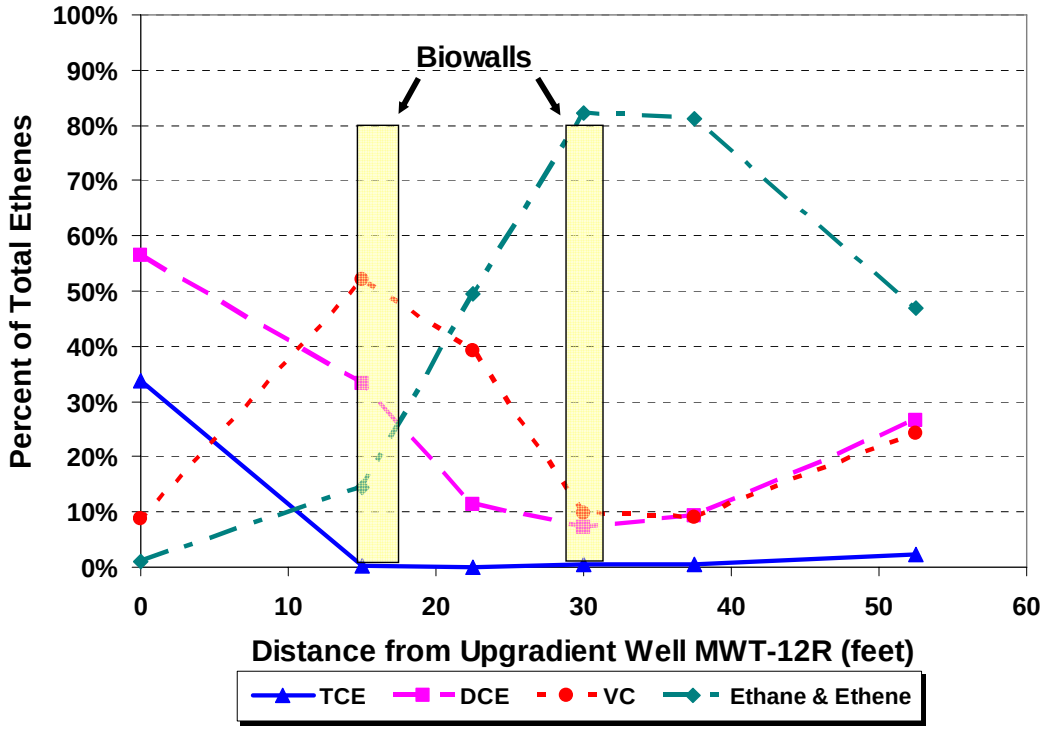

Figure 7.6 Molar Fractions of Chloroethenes and Ethene/Ethane for the Northern Transect at 27 Weeks

7.1.3 Degradation Rate Calculations

If biodegradation has been stimulated by addition of organic substrate in a mulch biowall or bioreactor, an increase in biodegradation rates should be observed within the treatment zone. Calculation of biodegradation rate constants prior to and after system construction may help demonstrate the effectiveness of the application. Degradation rate constant estimates can be calculated by many methods; USEPA (1998a) and Newell *et al*. (2003) provide examples and discussion for estimating biodegradation rate constants. As an example, Ahmad, *et al.* (2007b) describe the use of a steady-state analytical model based on the advection-dispersion equation developed by Van Genuchten and Alves (1982) to calculate first-order degradation rates from biowall case studies (**Table 3.2**).

In practice degradation rates are difficult to determine because enhanced bioremediation systems are seldom in a state of equilibrium. The addition of an organic substrate causes significant changes in the geochemical conditions and biological activity of the aquifer, which rarely stabilize over the treatment duration. Most methods to calculate degradation rates assume that steady state conditions (hydraulic, biogeochemical, and contaminant) exist (*e.g.,* the method of Buscheck and Alcantar, 1995).

However, a reasonable approximation of degradation rates may be calculated if geochemical and microbiological conditions stabilize to a moderate degree. To be considered "stable", important indicators of biogeochemical conditions (*e.g.,* pH, ORP, DO, sulfate, methane) and contaminant biodegradation (*e.g.,* contaminant molar ratios) should be reasonably constant over two or more sampling events over a period of perhaps 6 to 12 months.

Note that the time to reach quasi steady-state, or "stable" conditions may be dependent on the number of pore volumes displaced within the biowall, which in turn may be a factor of groundwater flow rate, hydrolysis rate of the mulch, and rate of utilization of DOC. Steady-state conditions (or peak performance) may be lost more quickly in aquifers with high specific discharge as the hydrolysis rate of the mulch eventually diminishes.

Degradation rates can then be estimated by adjusting the rate constants in an analytical model such as BIOCHLOR (Aziz *et al*., 2000; 2002) until model simulations provide an approximate match of average concentrations for monitoring locations upgradient, within, or immediately downgradient of the biowall or bioreactor. Accurate estimates of hydraulic gradient, hydraulic conductivity, and effective porosity are also required for calibration of an analytical model.

Procedures involving the use of BIOCHLOR to extract rate constants for reactions-in-series are also described in Ahmad *et al.* (2007b) for transects along the groundwater flow direction. A model like BIOCHLOR should be used with caution, as it is unlikely that all individual contaminant degradation rate constants will be first order or remain constant over time. This is primarily due to microbial adaptation, and changes in rates of mulch hydrolysis or depletion of readily degraded organic carbon. Both the use of the analytical model outlined by Ahmad *et al*. (2007a) based on Van Genuchten and Alves (1982) and the use of BIOCHLOR should be considered to yield only approximations of the rate constants.

A simpler method for parent compounds (*e.g.*, perchlorate) may be to calculate an average degradation rate based on the concentration of the contaminant entering and leaving the treatment system (usually determined by a monitoring location within or immediately downgradient of the treatment zone), and using an average contaminant residence time. This requires the hydraulics of the system to be well characterized, as well as consideration of the sorptive properties of the contaminant (*i.e.*, retardation). Calculation of residence time is discussed in **Section 3.5.2**.

Accurate estimation of biodegradation rate constants can be complicated by partitioning of chlorinated solvents between native sediment, the mulch mixture, and aqueous phases. Downgradient of the biowall or bioreactor the relative change in aqueous concentration may not be an accurate indicator of the destruction of mass achieved due to transfer of mass from the sorbed phase to the aqueous phase by enhanced desorption or back-diffusion from low permeability sediments. For example, note the rebound in concentrations of *cis*-1,2-DCE downgradient of a biowall in **Figure 7.5**. Biodegradation rate calculations based on relative concentrations between upgradient and downgradient locations that do not take this into account will be conservative in reflecting the actual rate of degradation achieved in the biowall or bioreactor.

Conversely, transfer of mass from the aqueous phase due to sorption to the mulch or compost may cause an initial apparent attenuation in aqueous phase concentrations. In these cases, the sorptive properties of the contaminants can be used to estimate mass transfer due to non-destructive mechanisms to calculate a more representative biodegradation rate. However, after several pore volumes have flowed through the biowall or bioreactor media, sorption will approach equilibrium and further reductions in CAHs may be attributed to transformation processes.

In summary, it may take several months to years after biowall or bioreactor construction for biogeochemical and microbiological conditions to stabilize, if stabilization occurs at all.

Approximate estimates of degradation rates can still be developed using monitoring data collected once the system has acclimated to a reasonable extent (perhaps 6 to 12 months after installation). While these approximate rate estimates may be lower than actual long-term degradation rates, they are useful to confirm estimates used for design of the system configuration and dimensions. Degradation rates may increase over a period of several months to several years as the microbial community grows and adapts to strongly anaerobic conditions.

7.2 REDUCTIONS IN PLUME TOXICITY

Sequential reductive dechlorination can produce toxic intermediate dechlorination products that may persist in groundwater for extended periods. A common concern is that these intermediate dechlorination products, specifically VC, may pose an equal or greater risk to human health and the environment than the parent compounds. VC is a known human carcinogen and has been assigned a federal drinking water MCL of 2.0 µg/L. Concerns over production of VC production are therefore justified.

However, the potential for production of VC often over shadows the overall reduction of toxicity that may be achieved. The remediation selection processes specified by the USEPA requires that each candidate technology or approach be evaluated against nine criteria including long-term effectiveness and the reduction of contaminant toxicity, mobility, and volume over time (USEPA, 1988). Accordingly, toxicity reduction is an important metric for evaluating site remedies.

The relative toxicity of site groundwater may be estimated by calculating the number of ***"toxicity equivalents"*** in the most contaminated wells at a site (Downey *et al*., 2006). For the purpose of this evaluation, a toxicity equivalent is defined as the individual CAH compound concentration divided by its assigned MCL. The total toxicity is defined as the sum of the individual compound toxicity equivalents. For example, a well location that contains 500 µg/L TCE, 140 µg/L *cis*-1,2-DCE, and 50 µg/L VC would contain:

$$500/5 + 140/70 + 50/2 = 100 + 2 + 25 = 127 \text{ toxicity equivalents} \qquad (7\text{-}1)$$

For this calculation, the MCL is assumed to be proportional to the relative toxicity of each CAH; the MCL being the most common measure of relative toxicity. A relatively toxic compound (*e.g*., VC with a MCL of 2.0 µg/L) will yield a higher toxicity equivalent than a less toxic compound (*e.g*., *cis*-1,2-DCE with a MCL of 70 µg/L). This approach allows the reduction in the overall toxicity of the site to be quantified over time as the mix of CAHs changes.

Table 7.3 provides an example of baseline CAH concentrations and concentrations at two years after installation for biowall and bioreactor demonstrations at OU-1, Landfill 3, Altus AFB, Oklahoma. For the biowall application, the upgradient (baseline event) concentrations of CAHs were compared to the CAH concentrations within the biowall (final event). For the bioreactor site, baseline or pre-treatment groundwater concentrations were compared to average post-installation concentrations from two source area wells exhibiting the highest concentrations of CAHs. Data at two years post-installation were selected to allow time for biological processes to acclimate to substrate addition.

Table 7.3
Reduction in Groundwater Toxicity, OU-1, Altus AFB, Oklahoma

Contaminant	MCL (µg/L)	Concentration (µg/L)	Toxicity Equivalent (unitless)	Concentration (µg/L)	Toxicity Equivalent (unitless)	Percent Change (%)
		Baseline Sampling Round		**Two-Year Sampling Event**		
OU-1 Biowall, Altus AFB, Oklahoma (Upgradient versus Wells in Biowall)						
TCE	5.0	5,198	1,040	9.0	1.8	
cis-1,2-DCE	70	1,137	16	417	6.0	
VC	2.0	4.0	2.0	100	50	
Total			**1,058**		**58**	**-95%**
Landfill 3 Bioreactor, Altus AFB, Oklahoma (Source Wells SW5 and SW6)						
TCE	5.0	12,423	2,485	3.0	0.6	
cis-1,2-DCE	70	1,491	21	11	0.2	
VC	2.0	6.0	3.0	370	185	
Total			**2,509**		**186**	**-93%**

Notes: MCL = United States Environmental Protection Agency Maximum Contaminant Level.
µg/L = micrograms per liter
TCE = trichloroethene; *cis*-1,2-DCE = *cis*-1,2-dichloroethene; VC = vinyl chloride

Even though concentrations of VC increased by over an order of magnitude, the biowall and bioreactor sites at Altus AFB have achieved high levels of toxicity reduction ranging from 93 to 95 percent. Although VC was produced at these sites, the footprint of the VC plume has been confined to within the initial contaminant plume, with no migration of VC beyond the original footprint of the TCE plume. This observation suggests that VC has limited mobility in groundwater at this site; VC degradation may occur under both anaerobic and aerobic conditions (*e.g.*, Fogel *et al.,* 2005; Coleman, *et al.,* 2002a).

The overall reduction in toxicity achieved during enhanced in situ bioremediation is often overshadowed by the appearance of VC and the regulatory focus on this compound. Even when DCE and VC are present, large reductions in toxicity can be achieved without expansion of the contaminant plume. The reduction of contaminant toxicity is another useful criteria for evaluating the performance of enhanced *in situ* anaerobic bioremediation using biowalls and bioreactors.

7.3 CHANGES IN BIOGEOCHEMISTRY

The variability associated with collecting groundwater samples often makes precise definition or delineation of zones of differing redox potential difficult, and various lines of evidence should be weighed together to determine if the biowall or bioreactor has stimulated anaerobic conditions conducive to the degradation processes being targeted. The following subsections describe the changes in biogeochemical conditions that are commonly evaluated for permeable mulch biowalls and bioreactors.

7.3.1 Competing Electron Acceptors

Native electron acceptors may be preferred over contaminants during anaerobic biodegradation. For example, nitrate may be preferred over perchlorate by microorganisms capable of utilizing both nitrate and perchlorate as electron acceptors. Iron reducing and sulfate

reducing microorganisms may compete for molecular hydrogen as an electron donor, at the expense of dechlorinating microorganisms. In this case, the presence of sulfate and ferric iron may limit the reductive dechlorination of CAHs due to their consumption of electron donor.

Competition for electron donor and utilization of native electron acceptors is based on the energy derived by microorganisms from the reaction Aerobic microorganisms using DO as an electron acceptor gain the most energy, and DO is the first native electron acceptor to be depleted during biodegradation. After depletion of DO, anaerobic microbes will use nitrate as an electron acceptor, followed by manganese (Mn^{4+}), ferric iron (Fe^{3+}), sulfate, and finally carbon dioxide (methanogenesis). These parameters are measured to establish the prevailing redox conditions. In some cases, it is easier to evaluate the byproducts of the reduction process (*e.g.*, soluble di-valent manganese (Mn^{2+}), soluble ferrous iron (Fe^{2+}), or dissolved methane), which are readily measured in groundwater samples.

Dissolved Oxygen. DO is the most thermodynamically favored electron acceptor used by microbes for the biodegradation of organic carbon, whether natural or anthropogenic. Anaerobic bacteria generally cannot function at DO concentrations greater than about 0.5 mg/L. Therefore, DO must be depleted before anaerobic degradation processes will occur.

Nitrate. After DO has been depleted in the treatment zone, nitrate may be used as an electron acceptor for anaerobic biodegradation of organic carbon primarily via denitrification. For anaerobic dechlorination to occur, nitrate concentrations in the contaminated portion of the aquifer should be less than approximately 1.0 mg/L (USEPA, 1998a). Nitrate should also be depleted for efficient degradation of perchlorate.

Iron and Manganese. Fe^{3+} and Mn^{4+} present in mineral form are used as electron acceptors during anaerobic biodegradation of organic substrate. During this process, Fe^{3+} is reduced to Fe^{2+}, which is soluble in water. Similarly, $Mn^{4+}$is reduced to soluble Mn^{2+}. Fe^{2+} and Mn^{2+}concentrations can thus be used as indicators of iron and manganese reduction. Care must be taken when interpreting these data because they may be biased low due to co-precipitation with sulfides.

Sulfate. After DO, nitrate, manganese, and iron have been depleted in the microbiological treatment zone, sulfate may be used as an electron acceptor for anaerobic biodegradation. Sulfate reduction results in the production of sulfide. High concentrations of sulfate (greater than 100 mg/L) have not been observed to inhibit anaerobic reductive dechlorination in biowalls and bioreactors at Altus AFB, Oklahoma; anaerobic dechlorination of CAHs occurs simultaneously with sulfate reduction. However, high levels of sulfate may cause a large proportion of bioavailable substrate to be consumed during sulfate reduction and lead to earlier depletion of the substrate supply. A rebound or reemergence in the concentration of sulfate may indicate substrate depletion or indicate the edge of the reducing zone. Production of sulfide is also a necessary step in the formation of iron monosulfides for stimulating biogeochemical reduction of CAHs.

Methanogenesis. During methanogenesis, acetate is split to form carbon dioxide and methane, or carbon dioxide is used as an electron acceptor and is reduced to methane. The fastest rates of anaerobic dechlorination typically occur under sulfate-reducing or methanogenic conditions. However, highly elevated concentrations of methane (greater than 5 to 10 mg/L) also may indicate that organic substrate is being consumed by methanogens at the expense of microorganisms capable of degrading the target contaminants *(e.g., Dehalococcoides* species).

7.3.2 General Geochemical Indicator Parameters

Geochemical indicator parameters commonly measured during system monitoring include ORP, pH, alkalinity, and chloride.

Oxidation-Reduction Potential. The ORP of groundwater is a measure of electron activity and is an indicator of the relative tendency of a solution to accept or transfer electrons. Redox reactions in groundwater containing organic compounds (natural or anthropogenic) are usually biologically mediated, and therefore the ORP of a groundwater system depends on and influences rates of biodegradation. While the ORP of groundwater generally ranges from -400 mV to +800 mV Eh (voltage reading against a SHE), most biological processes operate only within a prescribed range of ORP. Therefore, characterizing the range of ORP of the reaction zone provides an indirect indicator of the redox reactions that may be occurring. Care should be taken when measuring ORP in the field to note the reference electrode used and whether the data have been converted to Eh (see **Section B.3** in **Appendix B**).

pH and Alkalinity. There is a positive correlation between zones of microbial activity and increased alkalinity. Increases in alkalinity result from the dissolution of carbonate minerals driven by the production of carbon dioxide produced by the metabolism of microorganisms. Alkalinity is important in the maintenance of groundwater pH because it buffers the groundwater system against acids generated during anaerobic biodegradation. Biodegradation of organic compounds may generate enough acid to impact the pH of the groundwater. Controlling the range of pH in the reaction zone may be necessary to maintain effective anaerobic dechlorination.

Chloride. During biodegradation of CAHs dissolved in groundwater, chlorine atoms are released into the groundwater, resulting in increasing chloride concentrations in groundwater in the contaminant plume. However, high background concentrations of chloride may mask the production of chloride due to anaerobic dechlorination. Therefore, chloride concentrations is generally considered as an secondary indicator parameter and not as direct evidence that CAHs have been dechlorinated.

7.4 DIAGNOSTIC TOOLS FOR ANAEROBIC DECHLORINATION OF CAHS

Other analyses used to evaluate microbial activity and the potential for anaerobic dechlorination of CAHs to occur includes dissolved hydrogen, metabolic acids (VFAs), and molecular analysis for specific microbial species.

Dissolved Hydrogen. Molecular hydrogen is the primary electron donor used in anaerobic dechlorination and is produced by fermentation reactions. Concentrations of dissolved hydrogen have been used to evaluate redox processes in groundwater systems (Lovley and Goodwin, 1988; Lovley *et al*., 1994; Chapelle *et al*., 1995). Significantly, nitrate-, iron-, sulfate- and carbon dioxide-reducing (methanogenic) microorganisms exhibit different efficiencies in utilizing the hydrogen that is being continually produced. For example, nitrate reducers are highly efficient in utilizing hydrogen and maintain very low steady-state hydrogen concentrations. These characteristic ranges are listed in **Table 7.4**.

Table 7.4
Range of Hydrogen Concentrations for a Given Terminal Electron-Accepting Process

TERMINAL ELECTRON-ACCEPTING PROCESS	DISSOLVED HYDROGEN CONCENTRATION		
	(nmol/L)	(atm)*	(ug/L)
Denitrification and Manganese Reduction	< 0.1	$< 1.3 \times 10^{-7}$	$< 2.0 \times 10^{-4}$
Iron (III) Reduction	0.2 to 0.8	$0.26 - 1.0 \times 10^{-6}$	$0.4 - 1.6 \times 10^{-3}$
Sulfate Reduction	1 to 4	$1.3 - 5.0 \times 10^{-6}$	$2.0 - 8.0 \times 10^{-3}$
Methanogenesis	5 to 20	$63 - 250 \times 10^{-6}$	$1.0 – 4.0 \times 10^{-2}$
Optimum for Anaerobic Reductive Dechlorination	**2 to 1l**	**$2.6 - 125 \times 10^{-6}$**	**$4.0 \times 10^{-3} – 2.2 \times 10^{-2}$**

Adapted from Lovley *et al*., 1994; Chapelle *et al*., 1995; and Yang and McCarty, 1998

* In gas phase in equilibrium with water containing dissolved hydrogen.

Conversely, sulfate reducers and methanogenic bacteria are progressively less efficient and maintain higher hydrogen concentrations. Because each TEAP has a characteristic hydrogen concentration associated with it, hydrogen concentrations can be an indicator of predominant redox processes. Dechlorinating bacteria also exhibit an efficiency in utilizing hydrogen. If hydrogen concentrations are greater than approximately 1.0 nmol/L, then rates of anaerobic reductive dechlorination should have environmental significance.

Metabolic Acids. Metabolic acids, or short-chain VFAs, are typically an optional monitoring parameter used for diagnostic purposes. Metabolic acids produced by degradation of the primary substrate indicate microbial activity as well as substrate distribution and sustainability. A lack of metabolic acids (less than 1.0 to 10 mg/L) usually indicates that additional substrate is required. A more common procedure is to use TOC or DOC as an indicator of substrate availability.

Molecular Screening for Dehalococcoides Species. Molecular biological tools (MBTs) include screening for *Dehalococcoides* organisms, which is a useful diagnostic tool to indicate whether complete dechlorination of chlorinated ethenes (PCE, TCE, *cis*-1,2-DCE and vinyl chloride) to ethene is likely to occur (Stroo *et al*., 2006). MBTs are most likely to produce useful results after the growth of anaerobic microorganisms has been stimulated through substrate addition. Several MBTs are commercially available for *Dehalococcoides* organisms. A recent publication by SERDP and ESTCP (2005) summarized the current state of research for MBT and provides a general overview of the various tools and their respective advantages and disadvantages.

The most widely used MBT technique involves screening for the *Dehalococcoides* 16S rRNA gene. Early field demonstrations of this semi-quantitative, genus-specific test are reported in Fennell *et al.* (2001), Hendrickson *et al.* (2002), and Major *et al.* (2002). Current versions of this test offer much more precise quantification (*e.g.*, Lendvay *et al.*, 2003; Lu *et al.*, 2006) which may assist with the estimation of dechlorination rates. While these 16S rRNA gene-based tests are highly effective in most cases, there is potential for both false negatives and false positives. False negatives arise because *Dehalococcoides* organisms may not be detectable in all areas of a site (*e.g.*, Fennell *et al.*, 2001). Field sampling techniques and the degree of aquifer heterogeneity should be carefully evaluated when making a determination that *Dehalococcoides* species are completely absent. False positives may arise because different *Dehalococcoides* populations have different substrate and growth requirements, thus *Dehalococcoides* organisms may be detected at a site but may not always be able to dechlorinate the contaminants of

concern. Also, gene-based tests count both live and dead microorganisms, so concentrations measured may not accurately reflect the viable *Dehalococcoides* population.

Recently, new MBTs have been developed to address the false positive conditions described above. Quantitative screening for genes associated with vinyl chloride reduction to ethene (*vcrA* and *bvcA* genes) indicates whether the *Dehalococcoides* population detected has the potential for complete dechlorination of chlorinated ethenes (*e.g.*, Sung *et al.*, 2006). Also, MBTs that quantify expression of the 16S and/or dehalogenase genes are becoming available to detect only actively dechlorinating *Dehalococcoides* organisms. Microbial Insights (www.microbe.com) and SiREM Laboratories (www.siremlab.com) are two leading providers of commercial MBT services for *Dehalococcoides* and other dechlorinating organisms such as *Dehalobacter*.

Other MBTs can be used to examine the total microbial community in the aquifer and/or test for multiple dechlorinating bacterial populations at once. These techniques are primarily based on 16S rRNA gene analysis and include terminal restriction fragment length polymorphism (T-RFLP), 16S rRNA gene cloning, and denaturing gradient gel electrophoresis (DGGE) (*e.g.*, Löffler *et al*., 2000; Richardson *et al*., 2002; Duhamel *et al.*, 2002). However, the detection of specific populations such as *Dehalococcoides* may be subject to false negatives if the population of interest is not predominant in the overall community. In subsurface environments amended with organic substrates, high concentrations of iron-reducing, sulfate-reducing, and fermentative populations may mask the detection of the relatively low concentrations of dechlorinating organisms. Thus, these techniques are most productively used on laboratory cultures with relatively low microbial diversity as opposed to field samples.

Compound Specific Isotope Analysis (CSIA). CSIA can also provide valuable insights into biodegradation activity at a site, particularly when results from traditional analyses may be confounded by issues such as dilution or sorption/desorption from mulch mixtures or aquifer solids. CSIA is an innovative technique which can indicate whether a compound has undergone a chemical or biological transformation rather than a physical process such as dilution or sorption. CSIA may also help to elucidate biodegradation pathways, which can provide valuable data at sites where multiple CAHs are degrading to vinyl chloride or other compounds of concern (*e.g.*, Hunkeler *et al.*, 2002; Bloom *et al.*, 2000). CSIA data can be used in conjunction with chemical concentration data to provide an additional line of evidence supporting results from MBTs and microcosm studies. North American providers of commercial CSIA services for aquifer samples include several leading universities as well as Microseeps (www.microseeps.com).

7.5 SUBSTRATE DEPLETION AND SUSTAINING ANAEROBIC DEGRADATION

Mulch, other organic substrates, and inorganic amendments used in biowalls or bioreactors may be depleted over time due to biological and biogeochemical processes. Little is known regarding the minimum or threshold concentration of organic substrate that is required to sustain anaerobic degradation processes (*e.g.*, biotic reductive dechlorination, biogeochemical transformation), particularly once anaerobic conditions have been stimulated and a mature microbial population is present. Concentrations of TOC/DOC are typically used as an indication of substrate strength, with concentrations of TOC/DOC greater than 20 mg/L commonly thought to be necessary to support anaerobic reductive dechlorination, based on a natural attenuation screening matrix published in USEPA (1998a).

However, because mulch and compost are solid substrates and provide an excellent growth medium, an arbitrary TOC threshold alone may not be a good indication that anaerobic degradation is being sustained. Furthermore, the level of TOC necessary to sustain the reducing environment necessary for anaerobic degradation may vary significantly based on the contaminant and site-specific groundwater geochemistry and soil chemistry. The level of TOC necessary to sustain reduction of perchlorate at sites with low nitrate concentrations is likely to be much lower than that necessary to sustain anaerobic degradation of CAHs at a high sulfate site.

As an example, **Figure 7.7** illustrates concentrations of TOC and sulfate over time for a well in the OU-1 biowall at Altus AFB, Oklahoma. Over a period of approximately 34 months, TOC declined from an initial concentration of 2,800 mg/L to as low as 28 mg/L, but appears to have stabilized between 20 and 80 mg/L. Background sulfate levels are approximately 2,000 mg/L, but sulfate has been reduced to as low as 9.5 mg/L within the biowall (data not posted), with a slight rebound to 190 mg/L in April 2005. More importantly, the percent reduction in TCE within the biowall relative to an upgradient monitoring location remains above 99 percent.

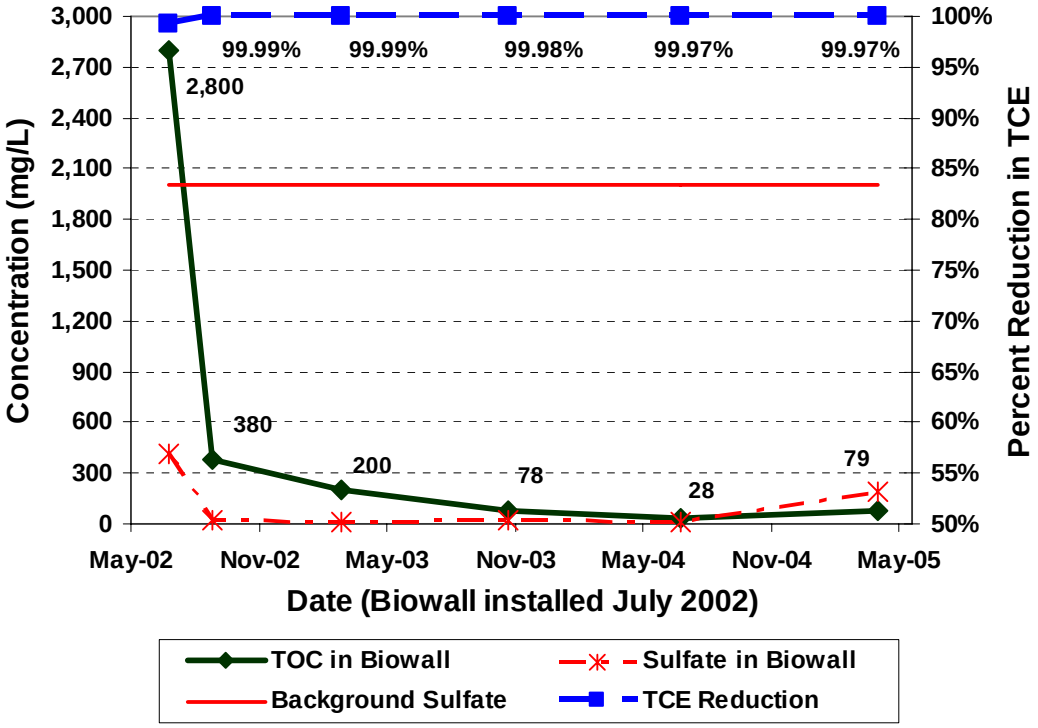

Figure 7.7 Total Organic Carbon, Sulfate, and Percent Reduction in TCE Over Time for Biowall Location MP-01, Altus AFB, Oklahoma

For a high sulfate site such as Altus AFB, both TOC and sulfate combined are likely to be key indicators for sustaining degradation of TCE. While TOC concentrations may decline to perhaps 10 to 20 mg/L, as long as sulfate remains depleted it is likely the OU-1 biowall will continue to effectively degrade TCE. An increase in ORP may be another good indication that reducing conditions are not being sustained. Because substrate depletion and sustaining anaerobic degradation are highly site specific, an evaluation of monitoring data over an initial period of 2 to 3 years should be used to develop a long-term O&M plan.

Another example of depletion of soluble organic carbon is illustrated on **Figure 7.8** for two biowall trenches at former NWIRP McGregor, Texas (data from EnSafe, 2005). Concentrations of TOC measured within the biowall trenches declined from over 300 mg/L to less than 12 mg/L at approximately 25 months after biowall construction. However, percent reductions in perchlorate remained greater than 99 percent, typically from over 400 to 500 µg/L to below detection limits. Because perchlorate may require less reducing conditions than sequential reductive dechlorination of chlorinated ethenes, the threshold concentration of DOC necessary to sustain perchlorate reduction may be lower.

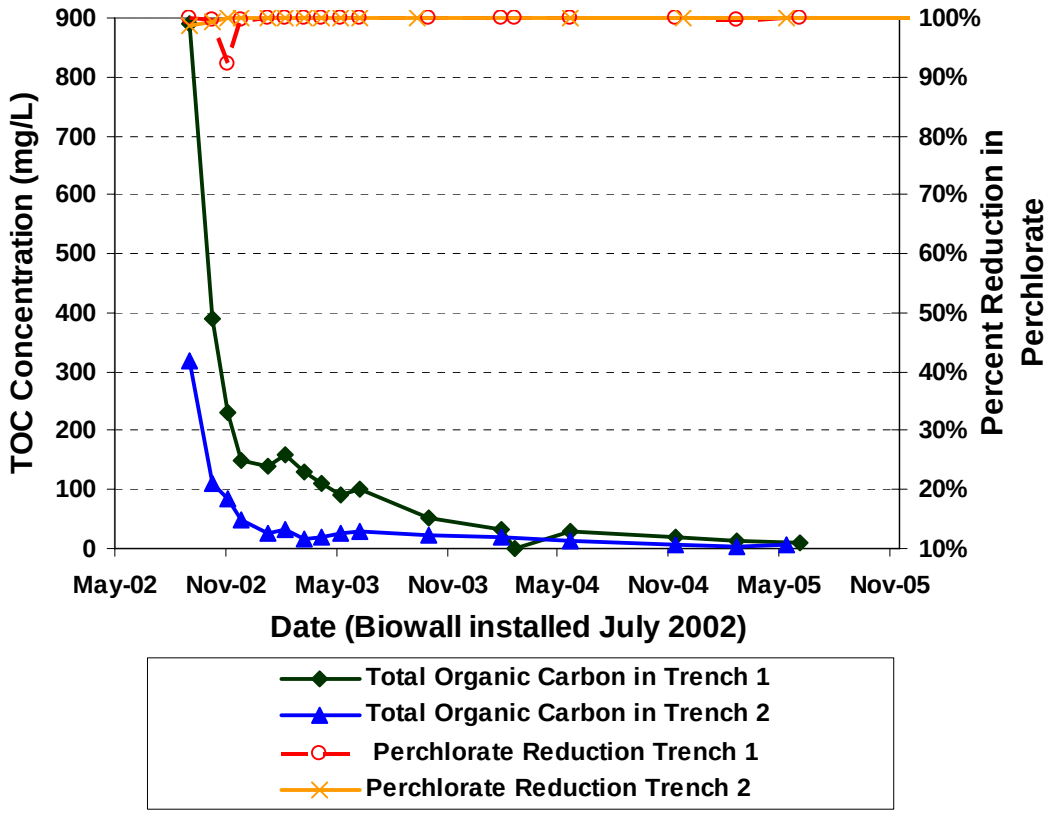

Figure 7.8 Total Organic Carbon and Percent Reduction in Perchlorate Over Time for Two Biowall Trenches at Former NWIRP McGregor, Texas

7.6 REPORTING OF SYSTEM PERFORMANCE AND COST

A report of system performance should be prepared after the initial operational period (perhaps after 18 to 24 months) that summarizes relevant site data collected during performance monitoring. This report should include a site-specific data review, a description of system installation, a detailed chronology, data collection and interpretation, and conclusions and recommendations. In particular, the report should clearly state the objectives and goals of the remedy and the extent to which they were achieved. Specific items to discuss in the report include the following:

Remedial Objectives (refer to **Section 2.1**):

- Overall remedial objectives and required regulatory compliance for the site.
- Specific field test and data quality objectives.

System Installation and Operation (refer to **Section 4**)**:**

- Description of biowall or bioreactor system construction and any operational or safety concerns.
- Source, volumes, and relative percentages of biowall materials and amendments.
- Results of any modifications made to the system design.
- As-built drawings, specifications, and catalog cut-sheets (*e.g.,* piping).
- Disposition of trenching spoils and description of site restoration.
- Cost summary.

System Performance (refer to **Section 7**):

- Anaerobic degradation of CAHs or other target contaminants (including downgradient extent) and apparent electron donor (organic substrate) requirements.
- Electron acceptor reduction and prevailing terminal electron accepting processes.
- Extent of contaminant mass destruction, including changes in contaminant concentrations and mass considering volatilization, dilution, degradation, and dechlorination product formation and persistence.
- Reaction kinetics and estimated degradation rates, including both biotic and abiotic processes, and a comparison to natural (background) degradation rates.
- Extent of sequential anaerobic dechlorination, including apparent accumulation of dechlorination products (*e.g.*, *cis*-1,2-DCE and VC).
- Extent of reduction in plume toxicity (Downey *et al*., 2006).
- System modifications required to optimize performance.
- Contributions or effects of any additional amendments added to the system (*e.g.*, replenishment using secondary substrates or microbial bioaugmentation).
- Any secondary issues such as impacts to secondary water quality, gas accumulation in the unsaturated zone, or impacts on site infrastructure and operations.

Recommendations

- Feasibility and relative cost-effectiveness of a permeable mulch biowall or bioreactor to meet full-scale remedial objectives.
- Scale-up issues, design considerations, and mitigation or contingency measures.
- Protocols and decision matrices for long-term O&M.

Based on this information, the report should detail the overall effectiveness of the treatment system and make objective recommendations regarding continued application of the biowall/bioreactor technology, and whether continued system operation or system expansion is warranted. The results of this evaluation are used to develop a long-term O&M plan as described in the following section.

SECTION 8

OPERATIONS AND MAINTENANCE PLAN

Biobarrier treatment systems may require operation for many years to perhaps decades without some form of source reduction. Therefore, the design life of the treatment system may outlast the longevity of the initial substrate materials. Monitoring of biowall systems to date indicates that the effective life span of a biowall system without substrate replenishment may vary from 3 to 5 years, or more. Replenishment of a biowall or bioreactor system involves the injection of a supplemental organic substrate, such as emulsified vegetable oil, in the biowall trench or bioreactor cell. The design life for an *in situ* bioreactor may similarly exceed the longevity of the initial substrate mixture. Therefore, an O&M plan with contingencies for substrate replenishment is useful to maintain biowall or bioreactor performance over periods of 3 years or more, although few formal O&M plans (*e.g.*, EnSafe, 2005) have been developed to date. Development of an O&M plan will be highly site-specific in regards to the hydrogeology, geochemistry, contaminants present, and biowall system configuration.

8.1 COMPONENTS OF AN O&M PLAN

An O&M plan should include the following:

- A summary of site conditions regarding groundwater flow, geochemistry, and contaminant concentrations;
- Specific performance objectives to achieve and maintain;
- An overview of the biowall or bioreactor system, including construction details and as-built drawings;
- Monitoring procedures and protocols;
- Criteria for determining when to enhance or optimize biowall performance; and
- Objectives and protocols for replenishing the biowall with supplemental organic substrate or other optimization procedures (*e.g.*, bioaugmentation or pH amendments).

The first three items are usually established in a work plan and construction completion report. A biowall performance study or evaluation is required prior to preparing the portions of an O&M plan that establish monitoring protocols, criteria for biowall replenishment, and replenishment or optimization procedures. This evaluation should be based on performance monitoring over the first few years of operation using the monitoring protocols described in **Section 6**. The objective of this initial evaluation is to determine the substrate and geochemical conditions that must be maintained to sustain biowall performance (**Section 8.2**), and the most

useful parameters that indicate when replenishment is required (**Section 8.3**). Protocols for amending a biowall (**Section 8.4**) can be developed once the criteria for determining when to replenish are established.

8.2 SUSTAINING BIOWALL PERFORMANCE

Application of enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls and bioreactors depends on development of optimal geochemical and redox conditions for anaerobic degradation processes to occur, and on sustaining appropriate levels of organic substrate in the reactive zone. The minimum or threshold concentrations of substrate that are required to sustain anaerobic degradation for a given biowall or bioreactor site are difficult to estimate given the current state of practice. Given this uncertainty, permeable mulch biowalls or bioreactors may fail to achieve long-term performance objectives or develop unanticipated long-term compliance problems. Therefore, determining substrate requirements to sustain degradation processes over a period of 3 years or more is a critical design and operational objective.

One objective of an O&M plan is to determine when replenishment is required prior to contaminant breakthrough. Therefore, the monitoring protocol for O&M should focus on critical geochemical thresholds and not simply on monitoring for breakthrough of the contaminants of concern. In addition, the frequency of monitoring should be adequate to provide sufficient time to implement a substrate replenishment event prior to unacceptable contaminant breakthrough. Thus, the frequency of monitoring will be a function of how accurate geochemical indicators are in determining when replenishment will be required. An iterative approach may be necessary, and O&M monitoring protocols should be evaluated periodically as additional data are collected and experience is gained with the treatment system.

8.3 PROTOCOLS FOR DETERMINING WHEN TO REPLENISH SUBSTRATE

Anaerobic degradation processes occur under specified reducing conditions. For example, efficient and complete anaerobic degradation of CAHs requires sulfate reducing or methanogenic conditions. On the other hand, degradation of perchlorate may only require manganese or iron reducing concentrations where nitrate has been depleted. Maintaining the appropriate redox conditions requires a minimal or threshold quantity of bioavailable organic carbon. Therefore, an appropriate analytical protocol for determining when replenishment is required is based on monitoring of the groundwater redox state and the amount of available organic carbon. A typical protocol may include some combination of the following:

- Contaminant concentrations (*e.g.*, CAHs, perchlorate, explosive compounds).
- Bioavailable organic carbon or electron donor supply: TOC or DOC, VFAs, humic and fulvic acids, or dissolved hydrogen.
- Indicators of native electron accepter demand: DO, nitrate, manganese, ferrous iron, sulfate, and methane.
- Indicators of redox state and chemical equilibrium: ORP and pH.

Most of these analytes are monitored during the initial performance monitoring period, and multiple lines of evidence should be used to determine when replenishment is necessary. But not

all of the parameters may be useful for determining when to replenish. For example, DO is often difficult to measure accurately in the field and may not provide useful information at sites which are naturally anaerobic (*i.e.*, DO is already depleted). As another example, analysis of VFAs, humic and fulvic acids, and dissolved hydrogen are relatively expensive compared to TOC. If TOC is an adequate indicator of substrate availability, then the other specialized analyses may be omitted from the O&M protocol.

A monitoring protocol was developed for treatment of perchlorate in groundwater at NWIRP McGregor, Texas, based on an optimization study (EnSafe, 2005). Parameters evaluated during the optimization study included perchlorate, DO, nitrate, methane, ORP, pH, TOC, VFAs, humic and fulvic acids, and dissolved hydrogen.

TOC was deemed to be the most useful parameter that indicated effective biodegradation of perchlorate. Depletion of TOC followed a first order rate, and the minimum range at which breakthrough occurred appeared to be between 5 and 10 mg/L. Native microbial populations that utilize perchlorate as an electron acceptor may prefer nitrate for metabolism. Perchlorate degradation was observed to be sensitive to the presence of nitrate (*i.e.*, diminished nitrate reduction due to a lack or organic carbon) at low concentrations of nitrate ranging from 0.1 to 0.5 mg/L.

ORP was also a useful indicator. Increases in ORP to greater than -50 mV were often the first evidence of impending perchlorate breakthrough, although this did not occur at all locations. Another parameter that appeared to be a useful indicator was the concentration of methane. Methane indicates highly reducing conditions, much more reducing than required to sustain perchlorate degradation. However, a decrease in methane to less than 2.0 mg/L appeared to indicate depletion of the biowall substrate and a good correlation was observed between a reduction in methanogenesis and inhibition of perchlorate degradation.

Given these observations, the parameters chosen for quarterly O&M monitoring included perchlorate, VOCs (where present), TOC, ORP, nitrate, methane, DO, and pH. DO and pH were retained primarily as stabilization parameters for well purging. A scoring matrix was established to determine when to replenish the biowalls. The scoring matrix included perchlorate, TOC, ORP, nitrate, and methane. TOC and perchlorate were weighted higher than the other parameters, methane was weighted the least. Other considerations included the number of sample locations indicating replenishment was required. For example, replenishment is initiated when two or more of four total sample locations in a biowall section indicate substrate amendment is needed.

In summary, multiple lines of evidence should be used to determine when replenishment is necessary. The replenishment protocol should account for temporal divergence from ideal conditions; confirmation of groundwater conditions over two consecutive monitoring events may prevent unnecessary or excessive amendment activities. The parameters most useful to determine when to replenish will be highly site-specific. Sites contaminated with CAHs will likely have a much different set of useful parameters than the example for perchlorate described above. For example, a cessation in sulfate reduction may be a critical indicator for sites where biogeochemical transformation is a predominant degradation pathway.

8.4 REPLENISHMENT OPTIONS

Replenishment of biowall or bioreactor systems involves the delivery of an organic substrate to the biowall trench or bioreactor cell. The primary options include substrate selection and the injection protocol.

8.4.1 Substrate Selection

Many fluid substrates can be injected into a biowall or bioreactor, including soluble substrates (*e.g.,* lactate, molasses, or fructose) and slow-release substrates (*e.g.,* emulsified vegetable oil and hydrogen release compound [HRC®]). Soluble substrates migrate with groundwater flow and require more frequent injection than slow-release substrates. Because of this, there are additional operational requirements and maintenance costs associated with soluble substrates. Unless it is desirable to impact a large portion of the aquifer downgradient of the biowall or bioreactor, slow-release substrates are likely to be more cost-effective based on operational requirements.

Emulsified vegetable oil is the substrate most commonly considered for biowall replenishment, for example at the former NWIRP in McGregor Texas. Biowalls at that location are being replenished every 3 to 5 years based on experience with perchlorate degradation (CH2M Hill, 2006). Emulsified vegetable oil is a suitable substrate based on 1) the ability to distribute the substrate throughout the biowall matrix, 2) the duration of which it will last and low frequency of injection required, and 3) lower product cost relative to other slow-release substrate types (*e.g.,* HRC® or HRC Advanced®). The use of vegetable oil and oil-in-water emulsions for enhanced bioremediation of chlorinated solvents is described in the AFCEE edible oil protocol (AFCEE, 2007).

HRC® products are another potential slow-release substrate. Typically the conventional product is not injected out more than a foot from the point of injection. Because there is little lateral (transverse) dispersion in a biowall trench, conventional HRC® may not be a suitable product because it would have to be injected on very close centers (less than 5 feet on center). In addition, conventional HRC® products cannot be injected using dedicated piping systems due to the high viscosity of the product. A newer product, 3-D Microemulsion (3DMe)™, a form of HRC Advanced®, may be a more suitable product for distribution in a biowall or bioreactor as it can be diluted prior to injection.

The final selection of substrate type should consider site-specific remedial objectives and system configuration. While a slow-release substrate may be the most appropriate for a passive biowall system, the use of soluble substrates may be more beneficial for systems with a recirculation component. For example, a soluble substrate may be periodically amended in-line with a recirculating bioreactor, where the treatment zone beneath and adjacent to the bioreactor cell is many times larger than the volume of the bioreactor itself.

8.4.2 Substrate Loading and Injection Volumes

The amount or concentration of substrate that is to be applied must be determined once a substrate has been selected. A common question is “how much” substrate to add, or what the substrate loading should be. Because biowalls are typically constructed perpendicular to groundwater flow and are less than 3 to 6 feet wide, there is little transverse dispersion within the biowall itself. For this reason the substrate should be physically distributed throughout the

entire biowall volume, requiring injection volumes equal to or greater than the effective pore volume of the biowall.

8.4.2.1 Substrate Loading

Methods to determine how much substrate to apply may be based on 1) mass discharge of contaminants and native electron acceptors, 2) a ratio of substrate to the mass of solid media in the biowall or bioreactor, or 3) an empirical concentration of substrate based on past experience. The theory of determining substrate requirements based on mass discharge of contaminant and native electron acceptors and stoichiometric relationships is described in Appendix C of AFCEE *et al*. (2004). Many product vendors offer similar spreadsheet calculations for determining how much of their product to use. An example of calculating substrate requirements for emulsified vegetable oil can be found in Appendix D of AFCEE (2007).

Often a significant design factor is applied when using calculations based on mass discharge and stoichiometric calculations. Due in part to this uncertainty, some practitioners may base substrate loading on ratios or percentage of substrate relative to the mass of solid media, or to the pore space of the treatment zone. These percentages or ratios are typically based on vendor recommendations or on past experience. For example, applying sufficient vegetable oil to account for 2 to 5 percent of the pore space (by volume) of the treatment zone should provide sufficient substrate to stimulate reductive dechlorination of CAHs for a year or more at most sites.

These type of estimates should still be compared to calculations based on mass discharge and stoichiometry to confirm that adequate substrate is being applied. Future replenishment applications and performance monitoring will help the practitioner understand the appropriate substrate loading to use for biowall and bioreactor systems; each site will be unique.

8.4.2.2 Substrate Volumes

To ensure that substrate is uniformly distributed throughout the biowall, the injection volume should be sufficient to displace at least one pore volume of the section of biowall being treated. Because the permeability of the biowall material should be much greater than the surrounding formation, substrate injected under pressure will tend to flow within and along the biowall trench. Although some substrate will flow into the surrounding formation, the total pore volume of the biowall section is a first approximation of the volume of the substrate mixture to inject.

The total volume to inject in each biowall section can be calculated by multiplying the biowall dimensions to obtain a total trench volume, then multiplying by the matrix porosity to estimate the trench section pore volume. For example, given a trench 100 feet long, by 2 feet wide, with a maximum saturated thickness of 10 feet, the volume of the saturated portion of the trench is:

$$\textit{Biowall Volume} = 100 \text{ feet (length)} \times 2 \text{ feet (thickness)} \times 10 \text{ feet (depth)} \quad (8\text{-}1)$$

$$= 2{,}000 \text{ cubic feet}$$

Then the pore volume may be calculated as:

Pore Volume = 2, 000 (cubic feet) x 0.40 (estimated matrix porosity) (8-2)

= 800 cubic feet

= 800 cubic feet x 7.48 gallons per cubic feet = 5,984 gallons

If the substrate loading specifies a concentration based on pore volume, the volume of substrate product to use can be calculated by multiplying the substrate loading concentration times the total pore volume. Assuming the substrate is emulsified oil, that the loading specifies 2 percent by volume of the pore space is filled with oil, and that the product is 50 percent oil by volume, then the amount of product required is calculated by:

$$\textit{Product Volume} = \frac{\text{800 cubic feet (pore volume) x 0.02 (loading strength)}}{\text{0.50 (percent oil)}} \quad (8\text{-}3)$$

= 32 cubic feet

= 32 cubic feet x 7.48 gallons per cubic feet = 239 gallons of product

If the substrate loading is specified in weight per unit volume of the trench (*e.g.*, pounds of vegetable oil per cubic yard of biowall), then the weight of the product to use is derived from the total volume of the biowall trench. The volume of product to mix must account for the amount of active ingredient in the product used. The product is still mixed with sufficient make-up water to meet or exceed the biowall pore volume to ensure uniform distribution.

8.4.3 Injection Procedures

The procedure for replenishment of a biowall includes 1) procurement and mixing of the substrate, 2) injection and monitoring of volumes and injection pressures, and 3) confirmation of uniform substrate delivery.

8.4.3.1 Substrate Mixing

Mixing of the substrate may be conducted using mixing tanks or in-line metering systems. Examples of mixing emulsified vegetable oil are described in AFCEE (2007). Make-up water for the substrate mixture should be native groundwater, preferably extracted from within or downgradient of the biowall. The high permeability of the biowall mixture should allow for high rates of extraction. Extraction or injection wells and piping may need to be surged and developed to provide adequate flow, and biofouling may be a concern.

Simultaneously extracting and injecting from alternating wells installed along the biowall trench is one option to enhance distribution of substrate within the biowall trench. In this case, it may be possible to amend the substrate mixture in-line using proportional feed equipment. For biowalls with horizontal perforated piping along both the bottom and top of the biowall, groundwater may be extracted from the bottom pipe, amended with substrate, and injected in the top pipe. Inflatable packers may be used to section off the horizontal pipe into more manageable injection segments.

Groundwater from within the biowall may be extracted and mixed within large mixing tanks (*e.g.*, 10,000- to 20,000-gallon frac or Baker® tanks), then reinjected into the biowall trench. Using the example for calculating injection volumes in **Section 8.4.2**, the total pore volume of the 100-foot length of trench is approximately 6,000 gallons. Assuming that groundwater can be extracted at 30 gallons per minute (gpm), it would take approximately 3.3 hours to collect sufficient volume to inject throughout the entire trench section.

8.4.3.2 Substrate Injection

Substrates may be injected via dedicated injection wells or perforated piping. Alternatively, biowall materials are readily penetrated by direct-push techniques and the substrate may be injected through direct-push probes without dedicated injection systems. Injection pressures should be limited to less than 1.0 pound per square inch (psi) per vertical foot of biowall over the injection interval to prevent displacement or upheaval of biowall material. In general, injection pressures should be kept below 10 to 15 psi. Groundwater levels in monitoring wells within and adjacent to the biowall should be closely monitored. In general, groundwater should not be allowed to mound above the upper limit of the biowall mulch mixture to prevent migration of contaminated groundwater or loss of the substrate mixture through the vadose zone, or to prevent potential day-lighting of groundwater or substrate mixture to the surface. Many biowalls and bioreactors are capped with a low permeability clay layer to prevent infiltration of surface water. The hydraulic pressure associated with mounding groundwater above the mulch mixture may potentially compromise this seal.

8.4.3.3 Confirmation of Substrate Delivery

Confirming the distribution of the substrate should include 1) documentation that the appropriate volumes of substrate were injected at each location, and 2) post-injection monitoring to document that target levels of TOC were obtained at each sample location. Sampling and analysis of TOC during routine O&M monitoring is one option for confirming substrate delivery. It may also be beneficial to install additional or temporary well points in select locations between injection wells or between injection piping, particularly where there are not sufficient locations in the system monitoring network to document uniform delivery. These locations only need be sampled and analyzed once following injection to confirm that substrate has been delivered throughout the biowall or bioreactor system as designed.

8.5 LONG-TERM MONITORING OPTIMIZATION

O&M monitoring protocols should be reviewed periodically to ensure the sample protocols are adequate to document that performance objectives are being achieved and sustained. Analytes that provide little useful information should be removed, and the frequency of monitoring reduced as experience and predictive capabilities of system performance increase. For example, peripheral sections of the biowall system with relatively low concentrations, and where performance criteria are consistently achieved, may require less frequent monitoring (*e.g.*, annual) relative to sections of the biowall system across the center of the plume where concentrations are higher (*e.g.*, semi-annual monitoring).

SECTION 9

BIOWALL SYSTEM COSTS

9.1 BIOWALL SYSTEM LIFE-CYCLE COST

The life-cycle cost of a biowall system can be broken down into capital construction and the cost to operate and maintain the system, including performance monitoring. Trenching is the single most expensive item for construction of biowall systems, primarily due to the cost to mobilize large and expensive equipment such as continuous one-pass trenchers. Operational costs are primarily for performance monitoring and replenishing or rejuvenating the biowall. Modifications/contingencies will also increase life-cycle costs.

9.1.1 Capital Construction

The primary cost for installation of permeable mulch biowalls is for the trenching subcontractor, which may account for up to 70 percent of the total cost for construction. Mobilization of specialized equipment is a large portion of the trenching contract, ranging from $10,000 up to $60,000 for interstate mobilization of a large continuous one-pass trencher. The cost per foot of trenching is highly scale-dependent, both in terms of the depth required and the length of the trench to be installed. For example, the cost for mobilizing a large continuous chain trencher from DeWind Environmental that can excavate a trench up to 35 feet deep and up to 3 feet thick is approximately twice as much as the cost for mobilizing a smaller trencher that can excavate a trench up to 2 feet thick and 25 feet deep. In some cases, a series of narrower biowalls may be a more cost-effective option.

Because a large portion of the trenching cost is for mobilization, there is an economy of scale in the cost per linear foot for installing longer trench systems. The trenching subcontractor cost per linear foot drops to approximately $200 per foot for biowalls longer than 1,000 to 2,000 linear feet.

Figure 9.1 plots the costs of various Air Force trenching subcontracts, along with some competing bids, using continuous one-pass trenchers. Note that these costs also include site preparation (grading), mixing of the substrate mixture, and grading the site level after installation. The cost do not include the cost of materials or construction oversight. The data are subdivided into 1) installations up to 25 feet deep using a variety of commercially available trenchers, and 2) for installations to 35 feet in depth using a large, unique trencher owned and operated by DeWind One-Pass Trenching. Trenching subcontractor costs to install biowalls using one-pass trenchers ranges from less than $200 to $1,000 per linear foot depending on length and depth. Once the linear footage increases to over 1,000 to 2,000 feet, costs for trenching drop to approximately $200 per linear foot. Due to the economy of scale, the cost of a small pilot test per linear foot will be high relative to a full-scale application.

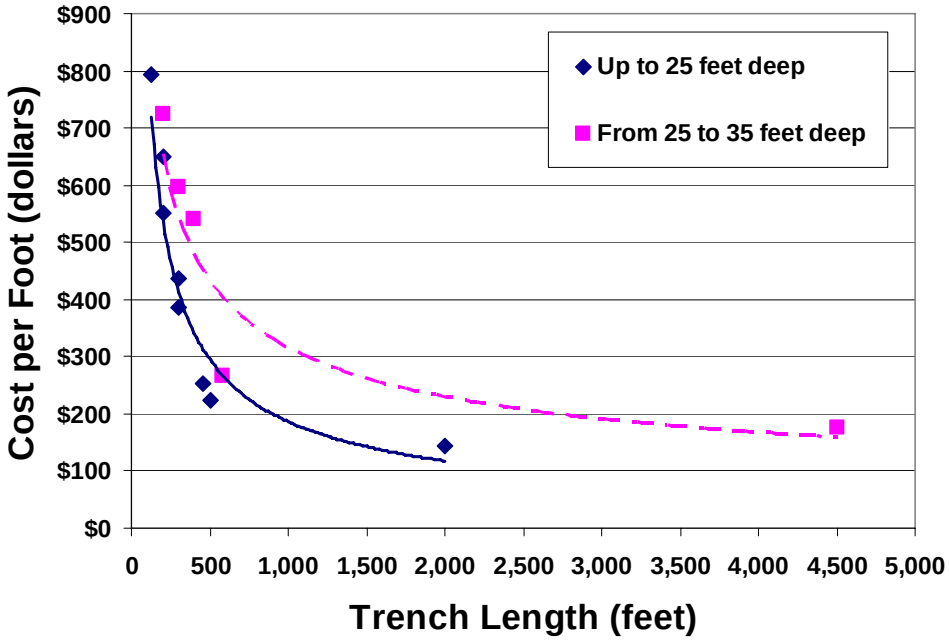

Figure 9.1 Trenching Cost per Linear Foot as a Function of Trench Length

The cost to install a trench using a conventional backhoe is much less, typically ranging from $50 to $100 per linear foot for depths less than 15 to 18 feet. Cost estimates to date for installing a biowall using long-arm excavators and biopolymer slurries have not been competitive with the one-pass trenchers.

Material costs are relatively inexpensive, on the order of 10 to 15 percent of total installation cost. Often free mulch can be obtained from a local municipality or land fill, but the mulch may need to be processed to smaller size and shipping to the site will need to be arranged. Therefore the cost of mulch is primarily dependent upon handling and delivery requirements, which may range up to $10 to $12 per cubic yard. Sand and gravel typically ranges from $12 to $20 per cubic yard delivered. The greater the distance from the source of the backfill material, the greater the cost of delivery.

Other factors that impact capital construction costs include permitting requirements, installation of piping or recirculation systems, additional amendments (*e.g.*, vegetable oil), surveying, installation of the monitoring network (number and depth of wells), and site restoration. In general, biowall trenches less than 500 linear feet (150 meters) can be installed for less than $300,000; including the cost of design, trenching, materials, installation of monitoring wells, and site restoration.

9.1.2 Operation and Maintenance

O&M over the first few years after biowall construction consists primarily of performance monitoring. Monitoring on a semi-annual basis is usually sufficient, although quarterly sampling is often stipulated by regulatory agencies. Monitoring costs are proportional to the size of the biowall system and the monitoring network. Annual monitoring and reporting costs may range from $20,000 per year for semi-annual sampling of a small biowall system with only one or two

well transects, up to perhaps $100,000 for quarterly monitoring of large scale applications with multiple biowall sections and monitoring transects.

After an initial performance evaluation, the monitoring protocol should be optimized for long-term operation. The optimized protocol should include only those monitoring locations and target analytes necessary to document that performance objectives are being achieved and to determine when system optimization (*e.g.*, replenishment) is required.

In addition to monitoring costs, long-term maintenance will likely require replenishment of substrate within the biowall system. An estimated cost to replenish a 300-foot length of biowall with a saturated thickness of 15 to 20 feet with emulsified vegetable oil is on the order of $30,000. There is an economy of scale in large replenishment applications due to a single mobilization of equipment and procurement of large quantities of bulk materials.

9.2 EXAMPLE BIOWALL SYSTEM COST

To illustrate the cost of a typical biowall application, costs are presented for the BG05 biowall at Ellsworth AFB, South Dakota (**Table 9.1**). A 580-foot long by 32-foot deep biowall was installed using a continuous one-pass trencher in June 2005. Total capital costs for system installation were less than $300,000, with the trenching subcontract accounting for over half of that amount.

Approximately $30,000 was spent on biowall materials, although approximately 25 percent of this cost was for iron ore and sulfate pellets added on a demonstration basis to a short 60-foot segment of the biowall trench. The iron ore (64 percent iron by weight) cost $5,800 for 6 cubic yards (15.4 tons) delivered. Sulfate was procured in the form of gypsum fertilizer pellets (16 percent sulfate by weight) from a local farm cooperative at a cost of $1,400 for 9.6 cubic yards (8,000 pounds) delivered.

The capital cost also includes work plan development, permitting, mobilization, installation of the monitoring network, baseline sampling, site restoration (grading and seeding), and a construction completion report. The capital construction cost may be compared to the cost of a ZVI wall, which for a 600-foot ZVI wall to a depth of 32 feet would cost over $1,000,000. Therefore, the biowall was installed for less than a third of the cost for a comparable ZVI wall.

The annual monitoring (two semi-annual events) and reporting cost is approximately $42,000 for this demonstration. This includes mobilization of a field crew, sampling three well transects of four to five wells each, and an extensive analyte list. Annual monitoring by a base contractor using an optimized and more streamlined monitoring approach would be closer to $30,000 a year for two semi-annual sampling events. In any event, the cost of monitoring is of consequence and may exceed capital construction cost over a period of 10 years or more.

Table 9.1
Biowall Technology Costs, BG05, Ellsworth AFB, South Dakota

Element	Cost ($)
Capital Costs	
Work Plan and Procurement	$19,300
Mobilization/Demobilization/Permitting	$9,600
Site Labor	$38,000
Equipment and Appurtenances	
- Monitoring Wells	$16,800
- Biowall Materials	$30,100
- Monitoring Equipment and Supplies	$3,200
Trenching Subcontractor	$154,600
Baseline Laboratory Analyses	$7,800
Surveying	$1,200
Reporting	$12,600
Total Capital Cost	**$293,200**
Annual Operating Costs (Performance Monitoring)	
Mobilization/Demobilization	$3,000
Site Labor (sampling)	$15,000
Sampling Equipment and Supplies	$4,000
Laboratory Analyses	$14,000
Project Management/Reporting	$6,000
Total Annual Operating Cost (per year, semi-annual sampling events)	**$42,000**

9.3 EXAMPLE BIOREACTOR SYSTEM COST

Installation and operation of the LF-03 bioreactor at Altus AFB, Oklahoma is described in **Appendix F.3**. The total cost for the technology demonstration was approximately $172,000; including $56,200 in startup and capital costs, $3,000 in O&M costs, and $112,800 in monitoring costs. Capital cost for constructing the Altus LF-03 recirculation bioreactor equates to approximately $56 per square foot. The construction cost also includes excavation of the bioreactor cell and disposal of excavated soil as non-hazardous waste to a landfill located on Altus AFB. Note that it is unlikely that a bioreactor would be cost effective relative to other *in situ* technologies if excavation and off-site disposal of soil as hazardous material is not already part of the site remedy.

The LF-03 bioreactor installation did not require disposal of hazardous soils, the excavated soil was placed as non-hazardous waste at a landfill on Altus AFB. It is unlikely that a bioreactor would be cost effective relative to other in situ technologies, such as direct injection of organic substrates, if excavation and off-site disposal of hazardous material is not already part of the site remedy.

Because of economies of scale in materials handling and placement, and in recirculation system construction, the design and construction cost for a 10,000-square foot recirculation bioreactor has been estimated at $22 per square foot, and for a one-acre (approximately 44,000 square feet) bioreactor at $12 per square foot (Parsons, 2006b). O&M costs are relatively standard for different bioreactor sizes. For example, estimated annual O&M costs for a 1,000-

square foot recirculation bioreactor (assuming semi-annual sampling) are $33,000, while annual O&M costs for a one-acre bioreactor are estimated at $50,000.

Recirculation bioreactors are likely to be limited to applications in CAH source areas of one acre or less, due to constraints on handling such large volumes of excavated material. The potential for excavated material to require disposal as a hazardous waste should be accounted for. Alternative designs may be considered for landfill source areas. If there is a high percentage of organic material in the landfill waste and there are no plans for excavation, it may be possible to recirculate through a network of vertical wells or shallow horizontal piping without the need for excavation.

SECTION 10

SUMMARY AND FUTURE DIRECTIONS

10.1 SUMMARY OF PERMEABLE MULCH BIOWALLS AND BIOREACTORS

Biowalls using common mulch and compost substrates are cost-effective alternatives to permeable reactive barriers using zero-valent iron or anaerobic biobarriers using more costly organic substrates. Biowall performance has been demonstrated to be equal or similar to other *in situ* barrier techniques (*e.g.*, see **Appendix F.1**). Advantages of biowalls or bioreactors include the following (**Section 1.4.5**):

- **Barriers to Contaminant Migration.** Biowalls are effective for shallow groundwater plumes to depths of 30 to 35 feet in very low to moderate permeability or highly heterogeneous formations. The continuity of the trench reduces the potential for groundwater bypass due to preferential flow paths, or non-uniform distribution of substrate that may occur with delivery of fluid substrates using injection wells.

- **Source Area Treatment.** Bioreactors are an alternative treatment approach for source areas where source removal via excavation is being considered. Combined with recirculation of groundwater, a bioreactor may treat an area much greater than the limited extent of the bioreactor cell or infiltration gallery.

- **Regulatory Acceptance.** To date biowall systems have been installed at approximately 13 facilities in 11 states and in five USEPA regions, having overcome any state and federal concerns regarding their installation. Examples of biowalls used for regulatory compliance include the full-scale biowall system installed at Altus AFB, Oklahoma as an interim corrective action; and a biowall system at the Ash Landfill site at Seneca Army Depot Activity, New York that is part of the final remedy in the ROD (**Appendix F.1**). The Navy was able to transfer the entire NWIRP McGregor property to the City of McGregor in November 2006 with the approval of the TCEQ and the USEPA.

- **Inexpensive Substrates.** Mulch, compost, and sand are relatively inexpensive when purchased in bulk quantities. Tree mulch can often be obtained for the cost of shipping and handling alone. Amendments to stimulate abiotic processes such as calcium sulfate (gypsum), magnesium sulfate (Epsom salts), or iron sulfate are also common and inexpensive industrial or agricultural products.

- **Low Operation and Maintenance Requirements.** Mulch biowalls require little O&M other than periodic performance monitoring over the first few years of operation. Biowall systems may need to be replenished with fluid substrates such as emulsified vegetable oil on a periodic basis, perhaps every 3 to 5 years (**Section 8**), but the cost to replenish is low relative to the capital cost of construction.

- **Destruction of Contaminants *In Situ:*** Contaminants that are treated have the potential of being completely mineralized or destroyed. Destruction of contaminants *in situ* is highly beneficial because contaminant mass is not transferred to another phase, there is no secondary waste stream to treat, and potential risks related to exposure during remediation are limited.

- **Potential Application to a Variety of Contaminants:** In addition to CAHs, the technology may be applicable to a variety of other contaminants subject to anaerobic degradation processes. Multiple contaminants can often be treated simultaneously.

- **Modifications and Contingencies.** Biowall trenches and bioreactors can be modified to include perforated pipe or injection wells (during or after system installation) for addition of fluid substrates to supplement the supply of organic carbon, if necessary. Injection or recirculation systems may also be used to add amendments (*e.g.*, pH buffering agents or bioaugmentation cultures) on a contingency basis.

- **Treatment Train Options:** Biowall and bioreactor systems can be used in tandem, or with existing or alternative remediation systems (*e.g.*, source removal via excavation) to optimize performance.

The primary limitation of using mulch and compost substrates is the depth to which they can be emplaced in the subsurface. One way to overcome this limitation is to use recirculation to bring groundwater into contact with the mulch and compost matrix in a trench or excavation. The use of alternative configurations such as recirculation may become more common in the future, as described below.

10.2 FUTURE DIRECTIONS

Not all sites will be suitable for installation of biowall trenches that can intercept an entire contaminant plume. However, the use of alternative configurations can increase the number of candidate sites for this technology. There are an increasing number of applications where recirculation is being used to bring contaminated groundwater into contact with the mulch mixture. Bioreactors are one example of using recirculation to treat source areas. As another example, Smith and Morris (2007) and Morris (2007) describe examples of using biowall trenches as infiltration galleries, where surface or groundwater contaminated with perchlorate is recirculated from downgradient sumps or extraction wells back through the biowalls. Solar powered extraction pumps (*e.g.*, **Appendix F.3**) are one way to make recirculation systems energy efficient and practical in remote settings.

While permeable mulch biowalls and bioreactors have been primarily used to treat chlorinated solvents, there is potential to expand the use of this technology to treat other contaminants such perchlorate, explosive compounds, and metals. For example, Benner *et al.*, (1999) describe the use of an *in situ* biobarrier in an aquifer containing effluent from mine tailings to treat metals and acid mine drainage. The reactive biobarrier was constructed using a mixture of municipal compost, leaf mulch, wood chips, gravel, and limestone. Stimulation of sulfate reduction has resulted in precipitation of metal sulfides, which has substantially lowered concentrations of metals in groundwater.

One of the most promising areas for future development of the technology is the addition of amendments to the backfill material to stimulate both biotic and abiotic degradation processes,

allowing the practitioner to optimize performance based on the type(s) of contaminant present and the desired degradation pathway(s) to be stimulated. The AFCEE *Workshop on In Situ Biogeochemical Transformation of Chlorinated Solvents* (AFCEE *et al.*, 2008) highlights the potential for remediation of chlorinated solvents in groundwater by biogeochemical transformation processes, and summarizes future research needs. Biowall and bioreactor applications will play a prominent role in this AFCEE initiative because 1) biogeochemical transformation has often been observed to be a prominent degradation process in these systems, and 2) the backfill material can be readily amended with solid phase sources of iron and sulfate to stimulate the process. Future biowall or bioreactor applications will provide further opportunities to demonstrate the ability to stimulate and sustain biogeochemical transformation of chlorinated solvents.

10.3 RESOURCES

The Technology Transfer Outreach Office of AFCEE maintains technical resources on their web site at:

www.afcee.brooks.af.mil/products/techtrans/Bioremediation/BIOREMresources.asp

Documents available at this site include work plans and result reports on AFCEE biowall and bioreactor applications, as well as links to relevant research documents from other sources. Other technical resources on enhanced *in situ* anaerobic bioremediation may be found on the ESTCP web site (www.estcp.org), the Interstate Technology & Regulatory Council web site (www.itrcweb.org), and the Federal Remediation Technology Roundtable web site (www.frtr.gov/multisitereports.htm#bioremediation).

SECTION 11

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APPENDIX A

KEY PROJECT PERSONNEL

APPENDIX A: KEY PROJECT PERSONNEL

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APPENDIX B

SUMMARY OF DEGRADATION PATHWAYS

APPENDIX B: SUMMARY OF DEGRADATION PROCESSES

B.1 CONTAMINANTS SUBJECT TO ANAEROBIC DEGRADATION

The majority of biowalls and bioreactors installed to date are for remediation of chlorinated solvents. The most common chlorinated solvents released to the environment include tetrachloroethene (PCE, or perchloroethene), trichloroethene (TCE), 1,1,1-trichloroethane (1,1,1-TCA), 1,2-dichloroethane (1,2-DCA), and carbon tetrachloride (CT). These chlorinated solvents and their chlorinated degradation products fall into the categories of chloroethenes, chloroethanes and chloromethanes. Collectively, these compounds are referred to as chlorinated aliphatic hydrocarbons (CAHs). CAHs in groundwater are problematic because of their health hazards and their resistance to natural degradation processes. However, these are oxidized compounds that are susceptible to reduction under anaerobic conditions by either biotic or abiotic processes.

Other common groundwater contaminants that are subject to reduction reactions are also amenable to enhanced *in situ* anaerobic bioremediation using permeable mulch biowalls and bioreactors. Constituents that can be treated with this approach include the following:

- Oxidizers such as perchlorate and chlorate;
- Explosives and ordnance compounds (*e.g.*, nitroaromatics like 2,4,6-trinitrotoluene [TNT]);
- Nitrate and sulfate.
- Dissolved metals (*e.g.*, hexavalent chromium); and
- *Potentially* chlorinated pesticides (*e.g.*, chlordane), polychlorinated biphenyls (PCBs), and pentachlorophenol.

The following sections describe degradation processes for substrates used in permeable mulch and biowall applications, and the anaerobic degradation processes for chlorinated solvents, perchlorate, and explosive compounds.

B.2 DEGRADATION PROCESSES FOR MULCH AND COMPOST

Plant mulch is the most common organic substrate used in permeable biowalls and bioreactors, and the degradation of plant mulch materials is described below. Other substrates, such as vegetable oils, will degrade differently in the subsurface. The reader may refer to Appendix D of the AFCEE Edible Oil Protocol (AFCEE, 2007) for further information on degradation of edible oil and soluble substrates that may be used in a biowall or bioreactor system.

B.2.1 Degradation of Plant Mulch

Ahmad *et al.* (2007b) and GSI (2005) describe the composition of tree mulch and how it degrades in relation to using mulch for *in situ* anaerobic bioremediation. The following discussion is summarized in large part from these publications. Tree mulch is composed of

approximately 40 to 50 percent cellulose, which is a natural polymer of glucose molecules, with the chemical formula $(C_6H_{10}O_5)n$ where *n* ranges from several hundred for wood pulp to over 6,000 for cotton (Senese, 2005). In addition to cellulose, wood is primarily composed of hemicellulose (20 to 30 percent), and lignin (25 to 30 percent), with lignin being the component of plant cell material most recalcitrant to biodegradation (Richard, 1996).

The cellulose, hemicellulose, and lignin contents of most North American species of trees have been analyzed and documented in the literature (Petterson, 1984). Variations from published values for a given mulch might represent blends of woods from different types of trees, partial composting of the mulch, or both. The degradation order of biopolymers in mulch generally follows in order of hemicellulose greater than amorphous cellulose, greater than crystalline cellulose, greater than lignin (Winandy and Lebow, 2001). Lignin is recalcitrant to biodegradation under anaerobic conditions, and once installed in a biowall or bioreactor it can be considered inert. Therefore, a qualitative analysis of the relative bioavailability of organic carbon in a mulch source may be made if the origin (species) of the mulch is known.

Heartwood consists of the central core of the tree that has been hardened using insoluble resins by the tree because this section contains dead cells from past cell embolisms. Conversely, sapwood grows around the heartwood (tree-ring formation) and contains living cells that are porous, and will break down more readily in a subsurface setting. Therefore, the leaves and soft tissue of the mulch are more amenable to biodegradation.

Aerobic composting of tree mulch is one method to break down the plant cell walls and increases the bioavailability of cellulose in the material. Therefore, adding compost to the mulch mixture provides a source of readily degradable organic carbon in the form of cellulose. Compost has little or no hemicellulose (*i.e.*, xylans), the cross-linking molecule that binds cellulose microfibrils to each other and to the inert lignin content of mulch. The absence of hemicellulose allows the remaining cellulose in compost to be readily available for hydrolysis (GSI, 2005; Ahmad *et al*. 2007a). Compost also supplies the inoculum for increased bioactivity for mulch hydrolysis and biodegradation.

B.2.2 Fermentation Reactions and Molecular Hydrogen

Researchers have recognized the role of hydrogen as the direct electron donor in the anaerobic dechlorination of CAHs (Holliger *et al*., 1993; Gossett and Zinder, 1996; Smatlak *et al*., 1996; Ballapragada *et al*., 1997; Cupples *et al*., 2003). Laboratory cultures used to study direct anaerobic reductive dechlorination are typically mixed cultures, with at least two distinct strains of bacteria. One strain ferments the organic substrate to produce hydrogen, and another strain uses the hydrogen as an electron donor for anaerobic dechlorination. Other direct electron donors also may be used for anaerobic dechlorination, including acetate (He *et al*., 2002). However, many researchers believe that molecular hydrogen is the most important electron donor for anaerobic dechlorination of CAHs. The following sections describe the fermentation reactions that produce molecular hydrogen and how hydrogen is utilized as an electron donor.

Hydrogen concentrations also are indicative of the dominant terminal electron accepting process (TEAP) occurring in groundwater (Lovely *et al.*, 1994; Chapelle *et al.*, 1995). **Table B.1** lists the hydrogen concentrations within which each electron-accepting process is favored. For the most rapid and extensive reductive dechlorination to occur, redox conditions should be in the sulfate reducing to methanogenic range. ***Yang and McCarty (1998) report that the optimal concentrations of hydrogen for anaerobic dechlorination to occur range from 2 nanomoles***

per liter (nmol/L) (mid-range of sulfate reduction) to 11 nmol/L (mid-range of methanogenesis).

Table B.1 Range of Hydrogen Concentrations for a Given Terminal Electron-Accepting Process

TERMINAL ELECTRON-ACCEPTING PROCESS	DISSOLVED HYDROGEN CONCENTRATION		
	(nmol/L)	(atm)*	(µg/L)
Denitrification and Manganese Reduction	< 0.1	$< 1.3 \times 10^{-7}$	$< 2.0 \times 10^{-4}$
Iron (III) Reduction	0.2 to 0.8	$0.26 - 1.0 \times 10^{-6}$	$0.4 - 1.6 \times 10^{-3}$
Sulfate Reduction	1 to 4	$1.3 - 5.0 \times 10^{-6}$	$2.0 - 8.0 \times 10^{-3}$
Methanogenesis	5 to 20	$63 - 250 \times 10^{-6}$	$1.0 – 4.0 \times 10^{-2}$
Optimum for Anaerobic Reductive Dechlorination	**2 to 1l**	**$2.6 - 125 \times 10^{-6}$**	**$4.0 \times 10^{-3} – 2.2 \times 10^{-2}$**

Adapted from Lovley *et al* , 1994; Chapelle *et al.*, 1995; and Yang and McCarty, 1998

* In gas phase in equilibrium with water containing dissolved hydrogen.

Biodegradation at higher hydrogen partial pressures may require more electron donor, as a larger portion of available hydrogen would be used by methanogenic bacteria. However, this is compensated for by higher rates of dechlorination under methanogenic conditions and by providing a sufficient amount of organic substrate. In practice, the amount of substrate added and hydrogen produced does not appear to be detrimental to anaerobic dechlorination of CAHs.

B.3 NATIVE (INORGANIC) ELECTRON ACCEPTORS AND OXIDATION-REDUCTION CONDITIONS

Anaerobic degradation processes will only occur under appropriate geochemical and reducing conditions. In the presence of sufficient organic carbon, microbes will facilitate oxidation-reduction reactions that will deplete native electron acceptors. After depletion of dissolved oxygen (DO), anaerobic microbes will use nitrate as a terminal electron acceptor, followed by manganese (Mn^{4+}), iron (Fe^{3+}), sulfate, and finally carbon dioxide (methanogenesis). The oxidation-reduction potential (ORP) of groundwater is a measure of electron activity and is an indicator of the relative tendency of a solution to accept or transfer electrons. Therefore, measurement of the ORP of natural groundwater will reflect the electron transfer activity of the prevailing terminal electron accepting processes (oxidation-reduction reactions) that are occurring. **Figure B.1** illustrates the redox potentials at which common reduction half reactions for native electron acceptors occur, measured as Eh – the voltage measured relative to a standard hydrogen electrode (SHE).

The ORP of a groundwater sample can change significantly within a short time following sample acquisition and exposure to atmospheric oxygen, therefore this parameter is measured in the field using a flow-through cell during purging of a monitoring well. The standard reference for ORP is set at 0.0 millivolts (mV) for a SHE. However, the use of hydrogen electrodes in the field is not practical and field meter readings for ORP are typically measured against a silver/silver chloride (Ag/AgCl) reference electrode. Redox potentials for reactions listed in the literature (*e.g.*, Thauer *et al.*, 1977 and Bouwer, 1992) involving common groundwater electron acceptors are usually reported as Eh, which is defined as a voltage reading against a SHE.

The practitioner should be aware of the reference electrode used to measure ORP in the field when comparing field ORP measurements to redox potentials listed in the literature. Redox potentials measured with a Ag/AgCl electrode are approximately 200 mV less than the Eh value, depending on the fluid used to fill the Ag/AgCl electrode (for examples, go to http://www.consultrsr.com/resources/ref/refpotls.htm).

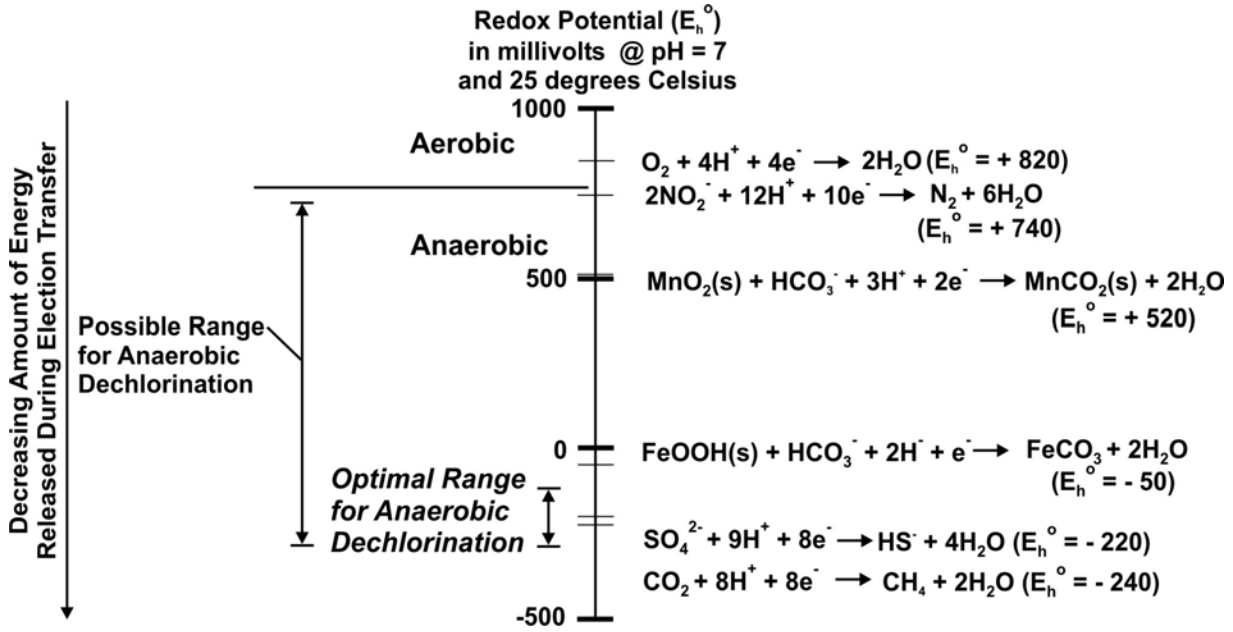


Figure B.1 Oxidation-Reduction Potentials for Various Electron-Accepting Processes
(modified from Bouwer, 1992)

As an example, the potential of Zobell solution used for calibration versus a Ag/AgCl electrode is +228 mV, which is the value typically used to calibrate the field meter. However, Zobell solution measured versus a SHE yields +448 mV (Eh). Some field meters using a Ag/AgCl electrode allow the user to specify +448 mV as the calibration value, in which case the meter will automatically compensate the Ag/AgCl reading to yield Eh measurements. ORP is also temperature dependent, which is usually not compensated for with field meters. Therefore, documentation of the type of field meter, electrode, calibration solution, and calibration procedure used, as well as the temperature of the groundwater during measurement, are essential to provide useful ORP readings. Manufacturers equipment manuals and tech notes are available to provide additional information (*e.g.*, go to https://www.ysi.com/portal/page/portal/YSI_Environmental/Support).

Measurement of ORP is further complicated in that ORP is a non-specific measurement, which means that the measured potential is reflective of the combination of all the effects of the dissolved species in groundwater. Therefore, ORP is only useful when combined with additional lines of evidence (*i.e.*, changes in concentrations of native electron acceptors between background conditions and the anaerobic treatment zone) to determine the predominant TEAPs that are occurring.

Estimated redox potentials (Eh) for reduction half reactions of chlorinated ethenes range from approximately 580 mV for PCE to TCE, down to 360 mV for *cis*-1,2-dichloroethene (DCE) to

vinyl chloride (VC) in aqueous solution at a pH of 7 and a temperature of 25 Celsius (^{o}C) (Vogel *et al.*, 1987). Redox potentials for reduction of chloroethanes are from 570 mV for TCA to DCA, down to 350 mV for chloroethane (CA) to ethane. Redox potentials for reduction of chloromethanes are from 670 mV for CT to chloroform (CF), down to 470 for chloromethane (CM) to methane. These are similar in range to chloroethenes (Vogel *et al.*, 1987). This range of redox potentials suggest that anaerobic reductive dechlorination may occur in the range of manganese reduction to iron reduction (**Figure B.1**). However, it appears that the most rapid and complete anaerobic dechlorination of CAHs occurs under the highly reducing conditions of sulfate reduction to methanogenesis (Bouwer, 1994). Therefore, as each sequential TEAP drives the ORP of groundwater downward, anaerobic dechlorination will occur more readily.

Prevailing redox conditions are largely a result of the relative amount of organic carbon (electron donor) and electron acceptors present. Thus, DO, nitrate, and bioavailable iron must be depleted before sulfate-reducing or methanogenic conditions can be induced. In general, USEPA (1998a) suggests that DO less than 0.5 milligrams per liter (mg/L), nitrate less than 1 mg/L, sulfate less than 20 mg/L, and total organic carbon (TOC) greater than 20 mg/L are favorable for anaerobic dechlorination of CAHs. In addition, ferrous iron and methane concentrations greater than 1 mg/L are indicative of favorable reducing conditions.

The optimal reducing condition for anaerobic dechlorination is generally lower as the oxidation state of the compound is lowered (*i.e.*, from PCE and TCE to DCE and VC). For example, anaerobic dechlorination of PCE and TCE to DCE may readily occur under iron-reducing conditions, but this redox condition may not be optimal for further degradation of DCE to VC and ethene. As a result, it is common for incomplete dechlorination to occur due when insufficient substrate is available to stimulate sufficiently reducing conditions.

B.4 DEGRADATION PROCESSES FOR CHLORINATED SOLVENTS

Understanding the processes and the pathways by which chlorinated solvents are degraded is essential to the application of engineered anaerobic bioremediation. To date, successful enhanced bioremediation has been accomplished through gaining an understanding of these naturally occurring attenuation processes and altering the environment to further stimulate them.

The most common chlorinated solvents released to the environment include PCE, TCE, TCA, 1,2-DCA, and CT. In general, the more highly chlorinated the CAH, the more oxidized the CAH is and the more susceptible it is to anaerobic or reductive degradation mechanisms. The relevant physical and chemical properties of chloroethenes, chloroethanes, and chloromethanes are listed in **Table C.1A** in **Appendix C**.

Less chlorinated compounds and/or dechlorination products such as DCE isomers, DCA isomers, VC, and CA are “cross-over” compounds in that they are also susceptible to oxidation reactions. This protocol is aimed at enhancing the anaerobic treatment of more chlorinated CAH parent compounds and their dechlorination products, but also provides practical guidance on how to evaluate other important removal mechanisms such as oxidation or abiotic reactions that can result in effective treatment throughout a larger *in situ* treatment zone.

Many CAHs can be cost-effectively degraded *in situ* by providing a source of biodegradable organic substrate. The application of enhanced *in situ* anaerobic bioremediation is covered in detail in *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents* (AFCEE *et al.*, 2004). As stated,

> "Site-specific conditions must be reviewed prior to selecting enhanced anaerobic bioremediation as a remedial alternative. The technology is not effective unless the contaminants are anaerobically biodegradable, strongly reducing conditions can be generated, a microbial community capable of driving the process is present or can be introduced, and an organic substrate can be successfully distributed in the subsurface."

In practice, the added organic substrates are first fermented to molecular hydrogen (H_2) and low-molecular weight fatty acids. These short-chain molecules (such as acetate, lactate, propionate, and butyrate) in turn provide carbon and energy to the microorganisms which facilitate reductive dechlorination.

In the reductive dechlorination process, microorganisms sequentially replace chlorine atoms with hydrogen forming more reduced dechlorination products. For example, the chlorinated ethenes are transformed sequentially from PCE to TCE to DCE to VC to ethene. If the microorganisms are able to obtain metabolically useful energy from reductive dechlorination, this process is referred to as dehalorespiration or halorespiration (USEPA, 2000b).

Other degradation processes may also occur. In some cases reductive dechlorination may be cometabolic, in which a CAH compound is reduced by an enzyme or co-factor produced during microbial metabolism of another compound in an anaerobic environment. In this case, biodegradation of the chlorinated compound does not yield any energy or benefit the growth of the microbe mediating the reaction (USEPA, 2000b). Anaerobic oxidation is a biologically-mediated reaction in which less chlorinated CAHs, such as *cis*-1,2-DCE and VC, are directly oxidized to carbon dioxide, water, and chloride. This reaction has been documented to occur under iron- and manganese-reducing conditions (Bradley and Chappelle, 1996 and 1997; Bradley *et al.*, 1998a and 1998b).

Abiotic or chemical dechlorination may occur where a CAH compound is reduced by a reactive compound that is not directly associated with biological activity. For example, this is the reaction targeted using zero-valent iron (Fe^0) in permeable reactive barriers. Note that addition of an organic substrate and creation of an anaerobic environment may create reactive minerals such as iron-monosulfides that can degrade CAHs (*e.g.*, Butler and Hayes, 2001). In this case the overall degradation pathway is referred to as ***biogeochemical transformation*** because the reactive mineral is formed in part due to biological processes. Other abiotic reactions that may be of significance include dehydrochlorination of 1,1,1-TCA to 1,1-DCE or hydrolysis of CA.

There are several potential reactions that may degrade CAHs in the subsurface, but not all CAHs are amenable to degradation by each of these processes (**Table B.2**). For example, PCE is not amenable to any aerobic degradation process, while TCE may be degraded by aerobic cometabolism that typically requires addition of a substrate in the presence of oxygen. However, anaerobic biodegradation processes may degrade not only PCE and TCE, but all of the common chloroethenes, chloroethanes, and chloromethanes. **Table B.3** further describes these potential degradation processes. Examples of the degradation pathways for chloroethenes, chloromethanes, and chloroethanes are shown on **Figure B.2**, **Figure B.3**, and **Figure B.4**, respectively (figures from AFCEE, 2007; provided courtesy of GeoSyntec Consultants).

Enhanced anaerobic bioremediation seeks to exploit anaerobic biodegradation processes to completely degrade chlorinated solvents to innocuous end products. This approach involves the addition of organic substrates to the subsurface to create anaerobic and microbiologically diverse reactive zones that are conducive to the anaerobic degradation of CAHs. The degradation processes, and the conditions under which they occur, are described in the following subsections.

Table B.2 Potential Degradation Processes for CAHs

	Compound [a/]											
	Chloroethenes				Chloroethanes				Chloromethanes			
Degradation Process	**PCE**	**TCE**	**DCE**	**VC**	**PCA**	**TCA**	**DCA**	**CA**	**CT**	**CF**	**MC**	**CM**
Aerobic Oxidation	N	N	P	Y	N	N	Y	Y	N	N	Y	P
Aerobic Cometabolism	N	Y	Y	Y	P	Y	Y	Y	N	Y	Y	Y
Anaerobic Oxidation	N	N	P	Y	N	N	Y	P	N	N	Y	P
Direct Anaerobic Reductive Dechlorination	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Cometabolic Anaerobic Reduction	Y	Y	Y	Y	P	Y	Y	P	Y	Y	Y	P
Abiotic Transformation	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Modified from Interstate Technology and Regulatory Council (ITRC) (1998), after AFCEE *et al*. (2004)

a/ PCE = tetrachloroethene, TCE = trichloroethene, DCE = dichloroethene, VC = vinyl chloride, PCA = tetrachloroethane, TCA = trichloroethane, DCA = dichloroethane, CA = chloroethane, CT = carbon tetrachloride, CF = chloroform, MC = methylene chloride, CM = chloromethane.

N = Not documented in the literature.

Y = Documented in the literature.

P = Potential for reaction to occur but not well documented in the literature.

B.4.1 Anaerobic Reductive Dechlorination

The process of microbially facilitated anaerobic dechlorination has been well documented, and discussions of the overall process are common in the literature (for example, see USEPA 1998a and 2000b). Anaerobic dechlorination of CAHs depends on many environmental factors including strongly anaerobic conditions, presence of fermentable substrates, generation of molecular hydrogen, and appropriate microbial populations to facilitate the reactions.

As listed in **Table B.2** and **Table B.3**, the three general reactions that may degrade CAHs by anaerobic reductive dechlorination include the following:

- **Direct Anaerobic Reductive Dechlorination** is a biological reaction in which bacteria gain energy and grow as one or more chlorine atoms on a CAH molecule are replaced with hydrogen in an anaerobic environment. In this reaction, the chlorinated compound

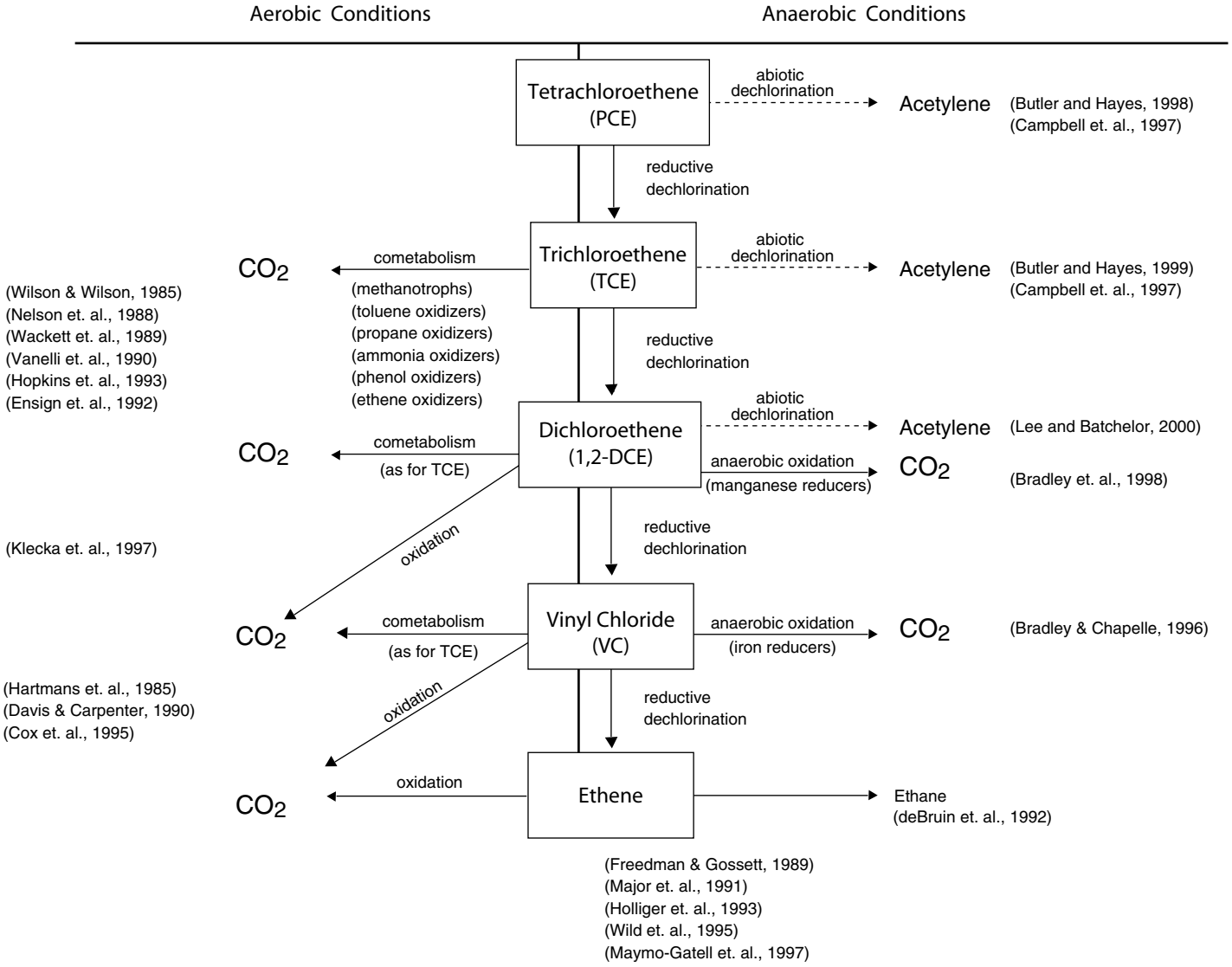
serves as the electron acceptor and hydrogen serves as the direct electron donor. Hydrogen used in this reaction is typically supplied by fermentation of organic substrates. This reaction may also be referred to as halorespiration or dehalorespiration (USEPA, 2000b).

Table B.3 Description of Degradation Processes for CAHs

Degradation Process	Reaction Process	Alternate Process Terminology	Example References
Aerobic Oxidation	Compound is oxidized (used as an electron donor). Yields energy to the microorganism facilitating the reaction.	Hydroxylation, Epoxidation	Bradley and Chapelle, 2000; Tandoi *et al*., 2001; Hage and Hartmans, 1999
Aerobic Cometabolism	Compound is oxidized by an enzyme or co-factor produced during microbial metabolism of another compound.	--	McCarty *et al.*, 1998; Hopkins and McCarty, 1995; McCarty and Semprini, 1994
Anaerobic Oxidation	Compound is oxidized (used as an electron donor) by microbes using electron acceptors other than oxygen. Yields energy to the microorganism facilitating the reaction.	--	Bradley and Chapelle, 1997; Bradley et al., 1998a, 1998b, and 1998c; Dijk *et al*., 2000
Direct Anaerobic Reductive Dechlorination	Compound is reduced (used as an electron acceptor). Yields energy to the microorganism facilitating the reaction.	Halorespiration, Dehalorespiration	Maymo-Gatell *et al*., 1999; Fennell and Gossett, 1998; He *et al*., 2003
Cometabolic Anaerobic Reductive Dechlorination	Compound is reduced by an enzyme or co-factor produced during microbial metabolism of another compound.	Anaerobic Cometabolism	Maymo-Gatell *et al*., 2001; McCarty and Semprini, 1994; Rheinhard *et al*., 1990
Abiotic Transformation	Compound is reduced by chemical reactions. For example, degradation by iron monosulfides and other reactive inorganic compounds.	Abiotic Reductive Dechlorination, Hydrolysis, Dehydrochlorination, Elimination, Hydrogenolysis, Dichloroelimination	Lee and Batchelor, 2002a, 2002b, and 2003; Butler and Hayes, 2001; Vogel and McCarty, 1987; Adrians *et al*., 2001; Gander *et al.*, 2002; Ferrey *et al*., 2004

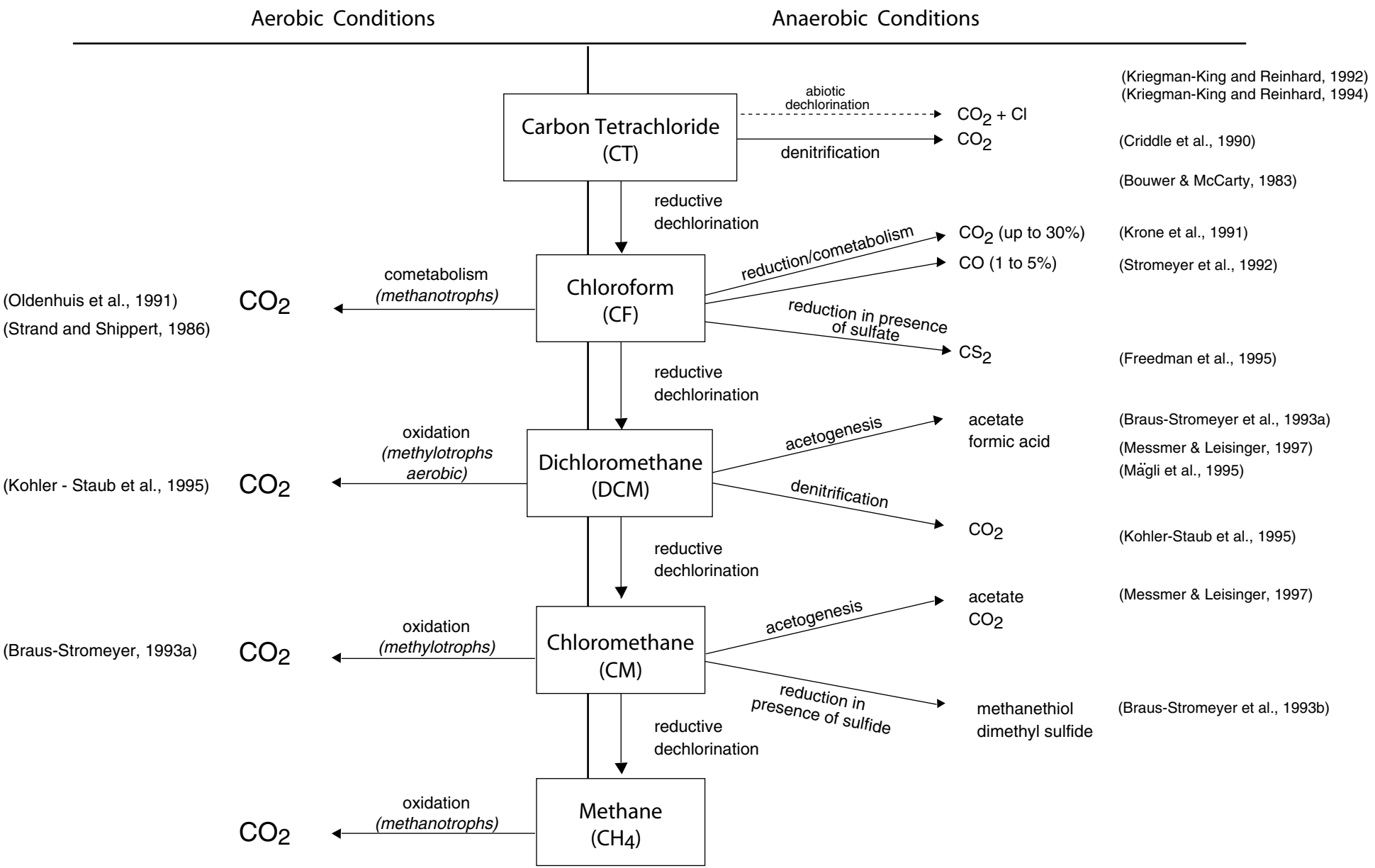
- **Cometabolic Anaerobic Reductive Dechlorination** is a reaction in which a chlorinated compound is reduced by a non-specific enzyme or co-factor produced during microbial metabolism of another compound (*i.e.*, the primary substrate) in an anaerobic environment. By definition, cometabolism of the chlorinated compound does not yield any energy or growth benefit for the microbe mediating the reaction (USEPA, 2000b). For the cometabolic process to be sustained, sufficient primary substrate is required to support growth of the transforming microorganisms.

- **Abiotic Reductive Dechlorination** is a chemical degradation reaction not associated with biological activity where a chlorinated hydrocarbon is reduced by a reactive compound (Vogel *et al*., 1987). For example, abiotic transformation of CT, TCA, PCE, TCE, and



Pathway chlorinated ethenes_0282.ai

Figure B.1 Pathways for the Degradation of Chlorinated Ethenes



carbon tetrachloride_pathway_0262.ai

Figure B.2 Pathways for the Degradation of Chlorinated Methanes

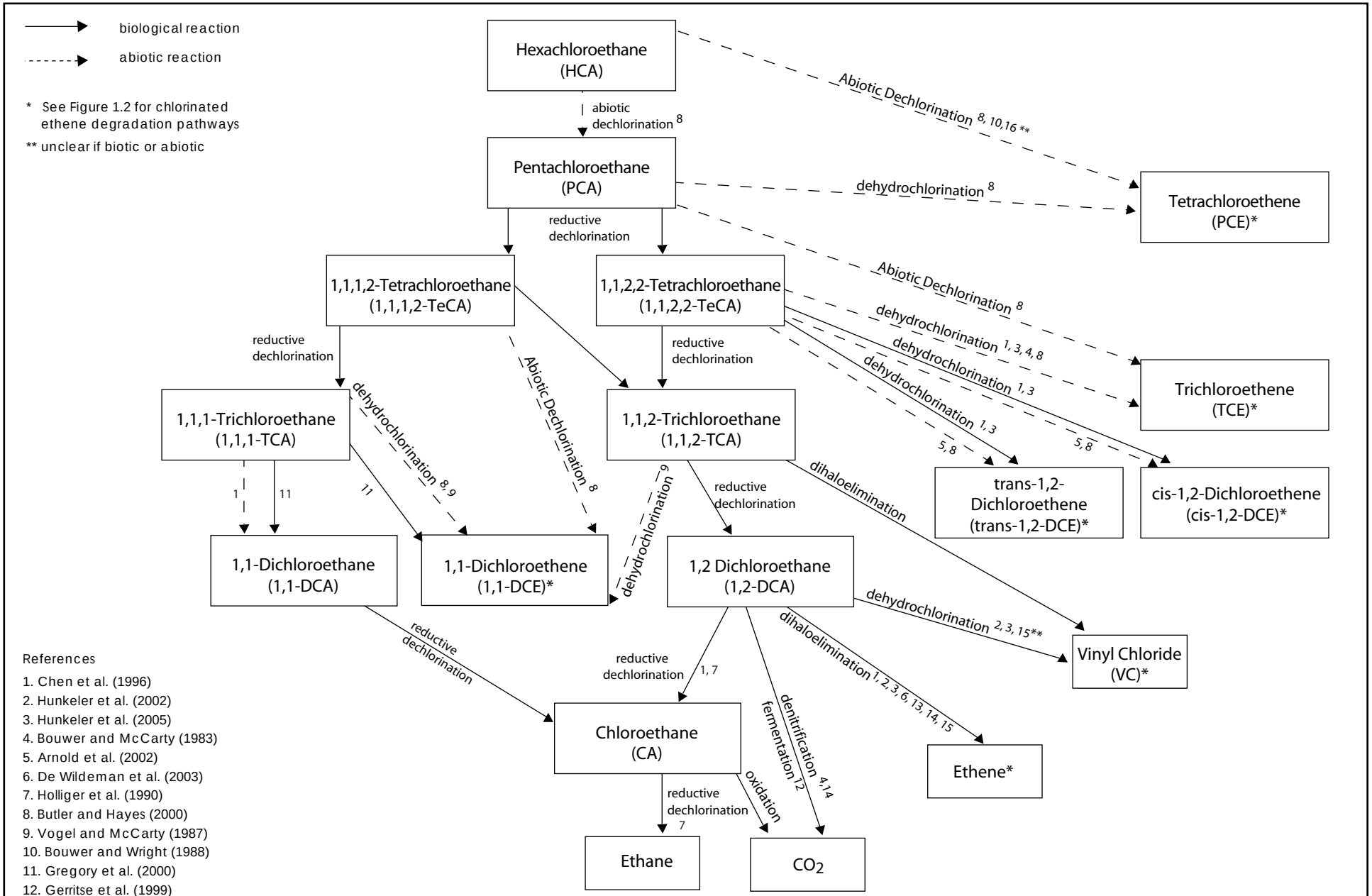


Figure B.3 Pathways for the Anaerobic Degradation of Chlorinated Ethanes

cis-1,2-DCE by metal sulfides has been investigated using pyrite (Weerasooriya and Dharmasena, 2001; Kriegman-King and Reinhard, 1994), troilite (Sivavec and Horney, 1997), mackinawite (Butler and Hayes, 1999 and 2000), and magnetite (Ferrey *et al.*, 2004). In this case, substrate addition may indirectly cause and sustain abiotic reductive dechlorination.

In practice, it may not be possible to distinguish between the three different reactions listed above at the field scale. As used in this document, *anaerobic dechlorination* includes the biotic processes of direct and cometabolic anaerobic reductive dechlorination, and abiotic reductive dechlorination.

Anaerobic reductive dechlorination of CAHs using hydrogen as an electron donor is typically based on the following two half reactions:

(B-1) $H_2 \rightarrow 2H^+ + 2e^-$

(B-2) $2e^- + H^+ + R\text{-}C\text{-}Cl \rightarrow R\text{-}C\text{-}H + Cl^-$

These half reactions can be combined and balanced to produce the following generalized complete reaction:

(B-3) $H_2 + R\text{-}C\text{-}Cl \rightarrow R\text{-}C\text{-}H + H^+ + Cl^-$

where C-Cl represents a carbon-chlorine bond in a chlorinated molecule, C-H represents a carbon-hydrogen bond, and R represents the remainder of the molecule. In these reactions, two electrons are transferred with molecular hydrogen (H_2) as the electron donor (which is oxidized) and the chlorinated molecule (R-C-Cl) as the electron acceptor (which is reduced).

Although fermentation products (*e.g.*, acetate) other than hydrogen have been identified as direct electron donors, several pure microbial cultures isolated to date require hydrogen as the electron donor for complete dechlorination of PCE to ethene (Maymo-Gatell *et al.*, 1997; Fennell and Gossett, 1998). Therefore, it appears that hydrogen may be the most important electron donor for anaerobic dechlorination.

In general, anaerobic dechlorination occurs by sequential removal of a chlorine atom. For example, the chlorinated ethenes are transformed sequentially from PCE to TCE to the DCE isomers (*cis*-1,2-DCE, *trans*-1,2-DCE, or 1,1-DCE) to VC to ethene. This process of sequential dechlorination is illustrated on **Figure B.4**.

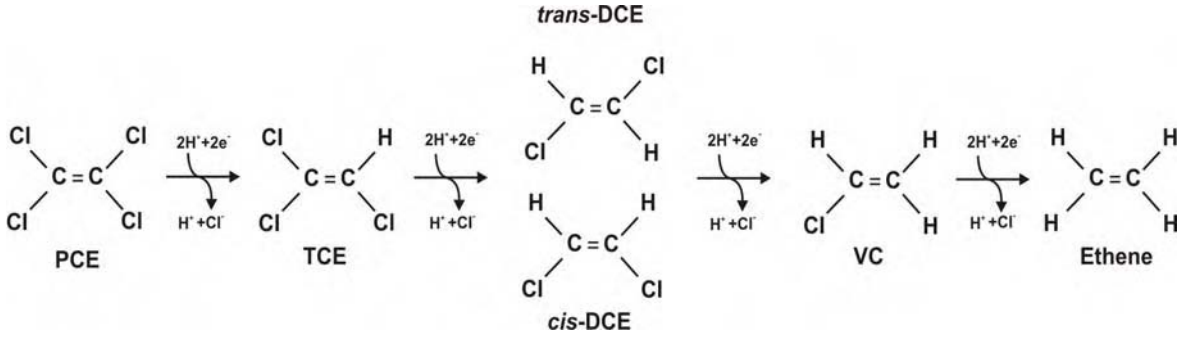


Figure B.4 Sequential Reduction of PCE to Ethene by Anaerobic Reductive Dechlorination

Similar analogies may be drawn for the chlorinated ethanes and chlorinated methanes, where potential accumulation of intermediate dechlorination products may occur. In general, the degradation pathways and microbiology of anaerobic dechlorination of chloroethanes and chloromethanes are less well studied than for the chlorinated ethenes. This is primarily because they occur less commonly as contaminants in groundwater. The common chloroethanes and chloromethanes may be transformed sequentially by anaerobic dechlorination as follows:

Chloroethanes: 1,1,1-TCA to 1,1-DCA to CA to ethane.

Chloromethanes: CT to CF to MC to CM to methane.

Sequential biotic anaerobic dechlorination of CAHs is associated with the generation of dechlorination products and chloride ions, and affects each of the chlorinated compounds differently. For example, of the chlorinated ethenes, PCE and TCE are more susceptible to anaerobic dechlorination because they are the most oxidized (*i.e.*, they may be degraded at higher redox potentials). In addition, PCE and TCE are more lipophilic than DCE or VC, and therefore tend to concentrate in microbial tissues. This may explain the fact that PCE and TCE are degraded first; these compounds are present at a higher abundance in the cells of the dechlorinating microorganisms.

Degradation of *cis*-1,2-DCE and VC requires more reducing conditions because they are the least oxidized of the chlorinated ethenes. Perhaps more importantly, they may degrade at lower reaction rates because they are less accessible to dechlorinating microbes. Therefore, the potential exists for *cis*-1,2-DCE and VC to accumulate in a treatment system when the rate at which they are generated is greater than the rate at which they are degraded. This is a common concern for VC because it is considered more toxic than the other chlorinated ethenes. However, there are other degradation pathways for VC (see **Table B.1**), and in the experience of the authors the formation and persistence of large VC plumes (larger than the footprint of the initial CAH plume) is rarely observed in practice

B.4.2 Alternate Degradation Processes

Multiple degradation pathways exist for CAHs in both aerobic and anaerobic environments (**Table B.1**). Microorganisms capable of anaerobic dechlorination of CAHs (*e.g.*, *cis*-1,2-DCE and VC) may not be ubiquitous or sufficiently abundant to be effective in meeting remedial objectives. However, there are other degradation pathways that may occur for these compounds. For example, **Figure B.5** illustrates two pathways for transformation of chlorinated ethenes.

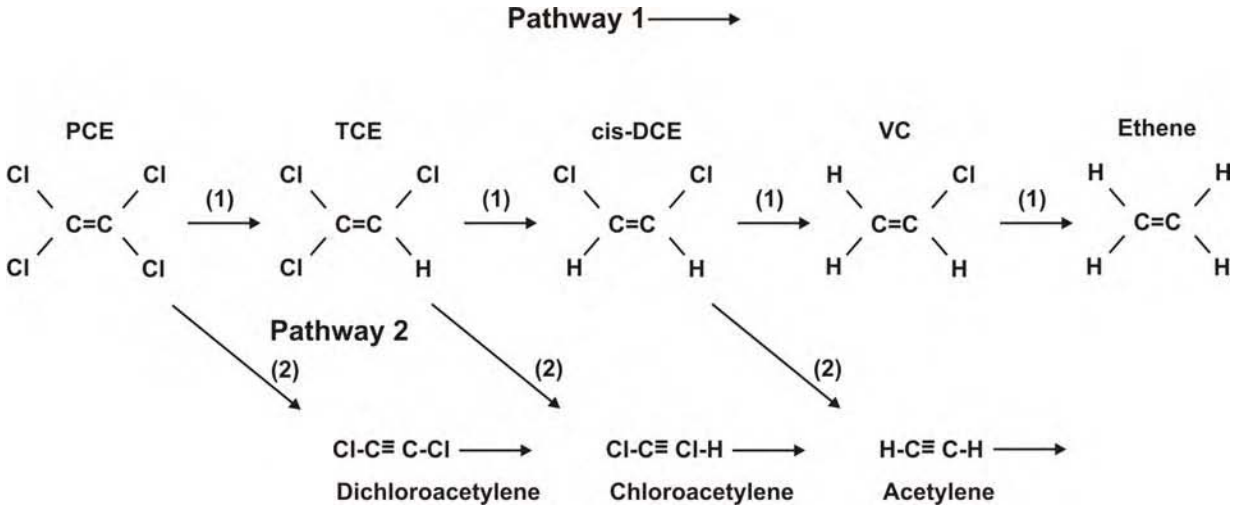


Figure B.5 Pathways for (1) Biotic Transformation of Chlorinated Ethenes and (2) Abiotic Transformation by Iron Monosulfide (modified from Butler and Hayes, 2001)

Figure B.6 (from AFCEE *et al.*, 2008) is a conceptual model of four potential biogeochemical processes documented in the literature. These processes include iron sulfide mediated transformation (Butler and Hayes, 1999 and 2000; Lee and Batchelor, 2002a), ferrous iron chemisorption mediated transformation (Williams and Scherer, 2004), green rust mediated transformation (Lee and Batchelor, 2002b), and magnetite mediated transformation (Ferrey *et al* , 2004). These processes show how both biological and geochemical reactions can be linked to result in the generation of reactive surfaces. These processes are just beginning to be understood.

Some of these alternative processes do not produce readily measurable or detectable dechlorination products (such as acetylene), and thus may be difficult to discern or quantify in the field. ***If measurable degradation of more highly chlorinated ethenes occurs without evidence of VC or ethene production, then these processes may be sufficient to achieve remedial endpoints.*** A lack of VC or ethene does not, by itself, provide adequate justification for bioaugmentation if degradation of contaminant mass (*e.g.*, oxidation of VC) is otherwise being achieved at acceptable rates.

B.4.3 Oxidative Pathways for CAHs

Oxidative pathways for CAHs occur when the CAH compound is used an electron donor in a coupled reaction with native electron acceptors. For aerobic oxidation, the electron acceptor is dissolved oxygen. However, oxidation of CAHs may also occur under anaerobic conditions, particularly where manganese (Mn^{4+}) or ferric iron (Fe^{3+}) are utilized as the electron acceptor (Bradley *et al.*, 1998b and 1998c). While oxidative pathways are not specifically targeted during enhanced anaerobic bioremediation, they may be important outside the anaerobic reaction zone in downgradient areas where groundwater geochemical conditions return to a natural state (redox recovery zone).

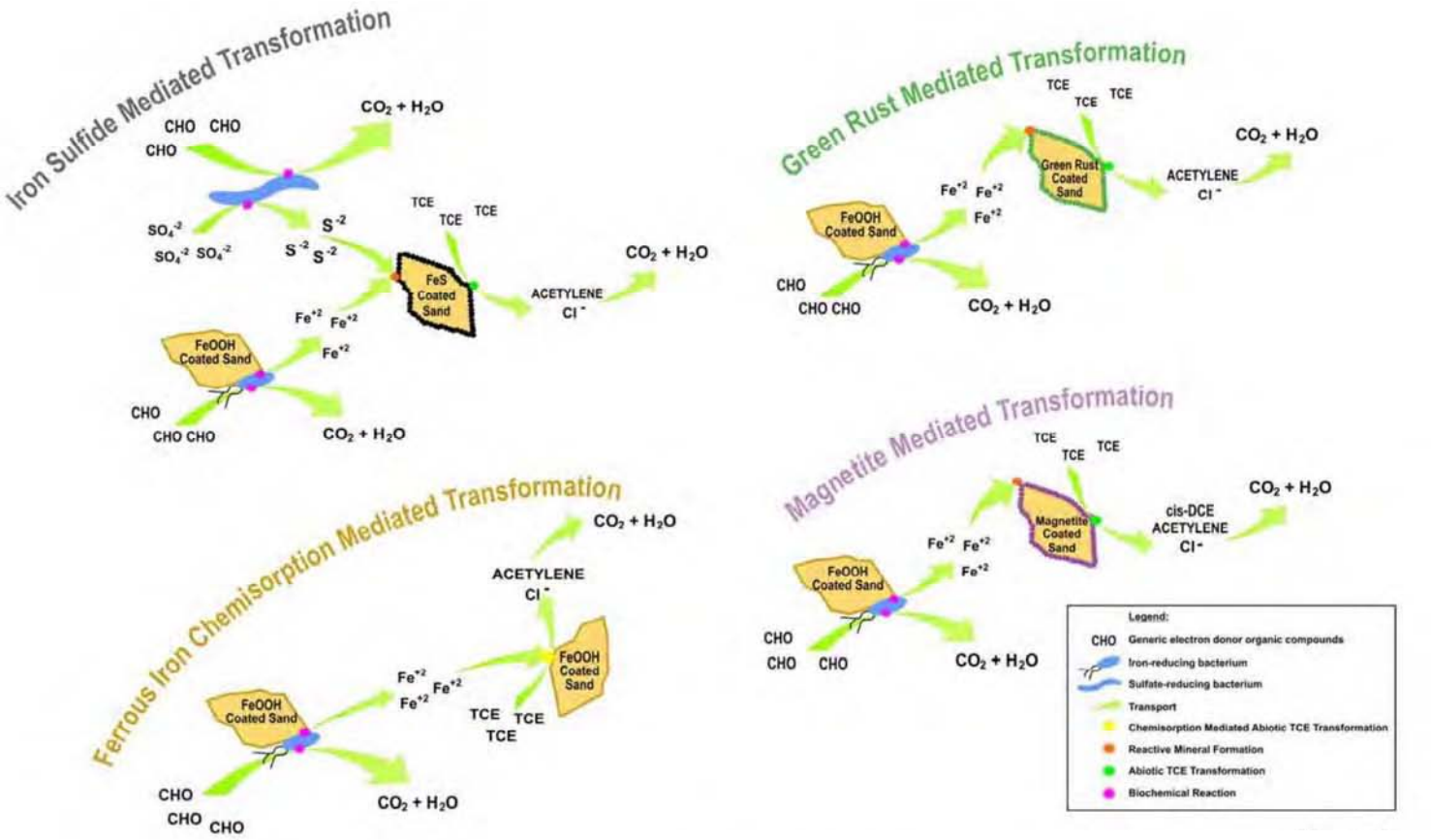


Figure B.6 Potential *In Situ* Biogeochemical Transformation Mechanisms (from AFCEE *et al.*, 2008)

B.4.3.1 Aerobic Oxidation of CAHs

Aerobic oxidation of highly chlorinated compounds such as PCE and TCE has been observed as a cometabolic process in the presence of a co-substrate such as methane (Wilson and Wilson, 1985; McCarty and Semprini, 1994). A variety of aerobic microorganisms in addition to methanogens have been identified that are able to oxidize chloroethene compounds to carbon dioxide (CO_2) without accumulation of regulated intermediate products (see Bradley, 2003 for a summary of the literature). However, most co-substrates are formed in the subsurface under anaerobic conditions, and sufficient quantities of both dissolved oxygen and co-substrate for cometabolic oxidation of CAHs are rarely present. An exception may occur during enhanced anaerobic bioremediation at the fringe of the anaerobic reaction zone where mixing with oxygenated groundwater may occur.

Less chlorinated compounds such as DCE and VC may be used as primary substrates for aerobic microbial degradation (Bradley and Chappelle, 2000). Aerobic oxidation of VC generally occurs at a higher rate than anaerobic reductive dechlorination. An example of a half reaction for the oxidation of VC is shown in the following equation:

(B-4) $$4H_2O + C_2H_3Cl\ (VC) \rightarrow 2CO_2 + 10H^+ + 10e^- + H^+ + Cl^-$$

In this case VC is an electron donor, yielding 10 electrons. This reaction is coupled to the reduction of oxygen (electron acceptor) as in the following half reaction:

(B-5) $$4e^- + 4H^+ + O_2 \rightarrow 2H_2O$$

Aerobic biodegradation of *cis*-1,2-DCE in the absence of primary substrates in a pure-culture, laboratory setting has been reported by Coleman *et al.* (2002b); however, it is less clear how significant this mechanism is for removal of DCE in the environment. Aerobic transformations of *cis*-1,2-DCE investigated under SERDP Project CU-1167 (personal communication with Dr. Frank Löffler) observed that aerobic oxidation of *cis*-1,2-DCE did not occur except under cometabolic conditions in the presence of VC, ethene, or methane. Conversely, Bradley and Chappelle (2000) and Coleman *et al.* (2002b) report that they were able to isolate microorganisms capable of utilizing *cis*-1,2-DCE as a sole carbon substrate for aerobic metabolism. Therefore, it is uncertain whether direct aerobic oxidation of *cis*-1,2-DCE in the environment is significant.

B.4.3.2 Anaerobic Oxidation of CAHs

Anaerobic oxidation of DCE and VC to CO_2 may occur under mildly reducing conditions such as iron- and manganese-reduction (Bradley, 2003). Bradley and Chappelle (1996) evaluated the microbial oxidation of VC in iron-reducing aquifer sediments. The addition of ferric iron (Fe^{3+}) to the anaerobic microcosms resulted in rates of VC mineralization comparable to those observed under aerobic conditions. Slower, but measurable, rates of VC mineralization were observed in microcosms under ambient iron-reducing conditions (Bradley and Chappelle, 1996; Bradley *et al.*, 1998b). Mineralization of VC to CO_2 decreased under increasingly reducing conditions, but was still observed under sulfate reducing and methanogenic conditions (Bradley and Chappelle, 1997; Bradley and Chappelle, 1998).

Additional microcosm studies indicate that DCE is also susceptible to microbial oxidation under anaerobic conditions (Bradley and Chappelle, 1998; Bradley *et al.*, 1998c), particularly under iron and manganese reducing conditions. Naturally occurring humic acids (common in mulch-based substrates) may also serve as terminal electron acceptors for microbial metabolism. Bradley *et al.* (1998b) demonstrated that both VC and DCE could be mineralized to CO_2 in the presence of humic acids.

Iron reduction is a common TEAP in permeable mulch biowall and bioreactor applications because Fe^{3+} minerals are common in the sand backfill material. Coupled with the production of humic acids from degradation of the mulch material, it would appear that anaerobic oxidation of DCE and VC may be an important process in the performance of mulch-based biowalls and bioreactors.

The ability of microorganisms to oxidize DCE and VC to innocuous products under anaerobic conditions is a potential alternative to the slow (relative to dechlorination of PCE and TCE), and difficult to predict, reductive dechlorination of DCE to VC and VC to ethene. The coupling of aerobic and anaerobic oxidation of VC may be one reason that VC plumes rarely expand beyond the initial extent of the PCE, TCE, or DCE plume present prior to the initiation of enhanced *in situ* bioremediation. Anaerobic oxidation of DCE and VC may also account for a lack of production of ethene and ethane where DCE and VC are produced and where a decrease in the concentration of total molar chloroethenes clearly indicates a loss of chloroethene mass.

B.4.3.3 Oxidation Processes in the Redox Recovery Zone

Oxidative pathways may be exploited in sequential anaerobic/aerobic systems where highly chlorinated compounds (PCE, TCE, or 1,1,1-TCA) are degraded by anaerobic dechlorination, and less chlorinated compounds such as VC or CA are aerobically degraded in a downgradient redox recovery zone or engineered oxidation system (*e.g.*, air sparging trench). In addition, more oxic groundwater zones provide for the precipitation of dissolved ions such as ferrous iron or manganese, or the oxidation of biogenic gases such as methane or hydrogen sulfide that are produced in anaerobic treatment zones. This will improve the aesthetic qualities of the groundwater such as taste and odor.

B.4.4 Abiotic Pathways

A number of abiotic processes may degrade CAHs, under both aerobic and anaerobic conditions. Abiotic pathways may include hydrolysis, elimination, dehydrohalogenation, hydrogenolysis, dichloroelimination, and abiotic reductive dechlorination by a variety of reactive minerals or compounds (**Table B.3**).

Hydrolysis is a substitution reaction in which an organic molecule reacts with water or a component ion of water, and a halogen substituent (*e.g.*, chlorine atoms in CAH molecules) is replaced with a hydroxyl (OH^-) group. This reaction often produces alcohols as products. For example, CA may undergo hydrolysis to ethanol (Vogel *et al.*, 1987).

Dehydrohalogenation is an elimination reaction involving halogenated alkanes (*e.g.*, chloroethanes) in which a halogen is removed from one carbon atom, followed by subsequent removal of a hydrogen atom from an adjacent carbon atom. In this two-step process, an

alkene (*e.g.*, chloroethene) is produced. For example, CA may be transformed to VC (Jeffers *et al.*, 1989).

Hydrogenolysis refers to the replacement of a chlorine atom (or other halogen) by a hydrogen atom in a process that may be either biotic or abiotic in nature. Dichloroelimination is the removal of two chlorines by a hydrogen atom accompanied by the formation of a double carbon-carbon bond.

Some abiotic process are not driven by redox processes (*e.g.*, hydrolysis and dehydrohalogenation), while other abiotic processes may be stimulated indirectly under the anaerobic conditions induced by addition of an organic substrate (*e.g.*, abiotic reductive dechlorination by reactive metal sulfides). Hydrogenolysis and dichloroelimination generally do not occur in the absence of biological activity, suggesting that biological activity somehow indirectly stimulates the reaction. Therefore, it is not clear whether these reactions are truly abiotic, or if they occur in a manner similar to cometabolism.

Many abiotic transformations of CAHs occur at rates that are too slow to have significance for environmental restoration of groundwater. Notable exceptions include hydrolysis and elimination of 1,1,1-TCA, and hydrolysis of CA and CM. Abiotic degradation of 1,1,1-TCA occurs by hydrolysis to acetic acid and elimination to 1,1-DCE. McCarty (1996) estimated that 80 percent of 1,1,1-TCA transformed by abiotic processes is converted to acetic acid and 20 percent to 1,1-DCE. 1,1-DCE is considered more toxic than 1,1,1-TCA, but also is subject to anaerobic dechlorination. Degradation rates for 1,1,1-TCA by hydrolysis have been reported with half-lives on the order of 1 to 3 years (Jeffers *et al.*, 1989; Vogel and McCarty, 1987). CA and CM are also subject to relatively rapid degradation by hydrolysis, with a reported half-life of 0.12 year for hydrolysis of CA to ethanol (Vogel *et al.*, 1987).

There appears to be a broad spectrum of metal-containing minerals that may cause abiotic degradation of contaminants in the subsurface. ***In situ biogeochemical transformation*** refers to processes where contaminants are degraded by abiotic reactions with minerals formed in the subsurface. These reactive minerals are thought to include reduced sulfide minerals such as iron monosulfide (*e.g.*, Butler and Hayes, 1999 and 2000), carbonate and sulfate green rusts (layered iron minerals) (*e.g.*, Lee and Batchelor, 2002b and 2003), or magnetite (*e.g.*, Ferrey *et al.*, 2004). In many cases these minerals are formed, at least in part or indirectly, from anaerobic biological processes. For example, chlorinated solvents such as PCE and TCE may be reduced in an abiotic reaction with iron monosulfide (FeS) that is formed in the subsurface under iron- and sulfate-reducing conditions. Alternatively, *cis*-1,2-DCE may be oxidized by reaction with magnetite, which could be a product of anaerobic biological ferric iron reduction. An advantage of these transformation reactions is that, in general, regulated intermediate dechlorination products are not produced.

For example, the presence of organic carbon, iron, and sulfate alone will typically result in the formation of reactive iron sulfide minerals (*e.g.* amorphous iron monosulfide, mackinawite, or pyrrhotite) due to the biological processes of iron and sulfate reduction (*e.g.*, Lee and Batchelor, 2002a; Butler and Hayes, 1999; Weerasooriya and Dharmasena, 2001). These sulfide minerals are also known as ***acid volatile sulfides (AVS)*** because, in contrast with pyrite (FeS_2) and elemental sulfur (S^o), they readily dissolve in hydrochloric acid. Other minerals of interest with respect to their capacities to support abiotic reductive dechlorination include, but are not limited to, pyrite, magnetite, and carbonate green rust (*e.g.*, Ferrey *et al.*, 2004; Sivavec and Horney, 1997).

The formation of these reactive minerals is of interest in that it may enhance overall contaminant destruction. For example, Shen and Wilson (2007) report a value for the abiotic degradation of TCE by FeS in biowall mulch material from Altus AFB, Oklahoma that is in the range of 0.5 to 2.3 per day per mole of FeS in contact with a liter of water. The minerals and associated abiotic degradation of CAHs may persist even if subsurface conditions are not sufficiently anaerobic to sustain rapid anaerobic biodegradation. Conversely, if organic carbon is depleted and native electron acceptor influx is high, these reactive minerals may be transformed to less reactive mineral forms. For example, FeS is oxidized to a ferric state such as pyrite (FeS_2).

B.4.4.1 Biogeochemical Transformation by Iron Mono-Sulfides

The occurrence of biogeochemical transformation of CAHs by FeS minerals may be pronounced for enhanced anaerobic bioremediation applications in high sulfate (*e.g.*, >500 mg/L) and high iron (*e.g.*, >5,000 milligrams per kilogram [mg/kg] iron in soil) environments. Because addition of an organic substrate may indirectly stimulate this process, practitioners should evaluate the potential for these reactions to occur in these environments.

As described in **Section 6.3**, samples of soil and biowall materials may be collected and analyzed for an assessment of the potential for biogeochemical transformation of CAHs in groundwater. Specifically, analysis of soil and mulch mixture chemistry may be performed to evaluate the presence of iron, manganese, and sulfides in aquifer sediments and biowall backfill material. Analyses include measurement of AVS, chromium extractable sulfide (CES), weak and strong acid extractable iron and manganese, and a bioassay test for bioavailable ferric iron.

Performance of these analyses is intended to facilitate assessment of the potential for the production of reactive metal sulfides and the abiotic reductive dechlorination of CAHs via ***biogeochemical transformation*** (Pathway 2 in **Figure B.5**). The process is termed "biogeochemical" because microbial processes facilitate geochemical conditions that cause the precipitation of FeS minerals in the aquifer matrix. The CAH compound is then reduced abiotically by the reactive FeS mineral.

Monitoring parameters that indicate that biogeochemical transformation of CAHs may be occurring include the following:

- Concentrations of parent compounds (*e.g.*, PCE, TCE, or CT) are reduced.
- Dechlorination products are ***not*** accumulating (*e.g.*, *cis*-1,2-DCE, VC, CA, or CM).
- A sufficient mass of FeS is present within the subsurface media to account for the reduction in CAHs observed (**Appendix D**).

The ***biological reduction*** of sulfate (SO_4^{2-}) coupled with the oxidation of organic material by sulfate reducing bacteria produces hydrogen sulfide (H_2S or HS^-), for example in the following reactions:

(B-6) CH_3COOH (*organic*) + SO_4^{2-} + $2H_2O$ ➔ $2CO_2$ + H_2S (g) +$2H_2O$ + $2OH^-$

(B-7) $2CH_2O$ (*organic*) + SO_4^{2-} ➔ $2HCO_3^-$ + H_2S (g)

Ferric iron in the subsurface soil or biowall material may be reduced to ferrous iron by either biological or chemical processes. The ***biological reduction*** of ferric iron to ferrous iron may proceed as follows (from AFCEE, 2002b):

(B-8) CH_2O (*organic*) + $4Fe^{3+}$(s) ➔ $4Fe^{2+}$(aq) + HCO_3^- + $5H^+$

Most ferrous iron (Fe^{2+}) will precipitate in mineral form, for example as FeS, iron disulfide (FeS_2), siderite ($FeCO_3$), or magnetite (Fe_3O_4). Fe^{2+} may also exchange with ions on clay minerals (Kennedy *et al.*, 1999).

Iron oxide or iron hydroxide minerals provide a strong chemical sink for H_2S forming various iron sulfide minerals. Hydrogen sulfide may ***chemically reduce*** ferric iron (Fe^{3+}) present in iron oxides or iron hydroxides to form FeS, for example with Goethite according to the following reaction:

(B-9) 2FeOOH (s) (*goethite*) + $3H_2S$ (aq) ➔ 2FeS(s) + S^o + $4H_2O$

These processes may occur when sufficient organic carbon, ferric iron, and sulfate are present. For the Altus biowall (**Appendix F.2**), organic carbon is present in the form of mulch and compost, ferric iron is present in the river sand used for biowall backfill and in the native sediments, and elevated concentrations of sulfate occur naturally in groundwater as a result of dissolution of gypsum and anhydrite from the aquifer matrix.

Precipitated iron sulfide mineral forms include amorphous iron sulfide (FeS), mackinawite (FeS), or pyrrhotite (FeS), but also greigite (Fe_3S_4) and others (AFCEE, 2002b). FeS minerals, which exist in a reduced state, may react rapidly with oxidized compounds such as TCE to form acetylene (Butler and Hayes, 1999). The suggested chemical expression for TCE dechlorination via FeS oxidation is:

(B-10) 4 FeS + 9 C_2HCl_3 *(TCE)* + 28 H_2O ➔

4 $Fe(OH)_3$ + 4 SO_4^{2-} + 9 C_2H_2 *(acetylene)* + 27 Cl^- + 35 H^+

Using this equation, four moles of FeS is sufficient to degrade nine moles of TCE. Based on the molar mass (grams per liter) of FeS (87.91) and TCE (131.39), it takes approximately 0.30 mg of FeS to degrade 1.0 mg TCE. However, the degree to which FeS is actually oxidized by reduction of TCE is uncertain (Kennedy and Everett, 2004). Sulfide (S^{2-}) from FeS may only be partially oxidized to a number of valence states up to S^{6+} (SO_4^{2-}). Therefore, excess FeS is needed to account for partial oxidation products. Further, some excess FeS will be required under field conditions to optimize contact with TCE and to facilitate oxidation of FeS by dissolved oxygen (O_2) or nitrate (NO^{3-}).

It is notable that the major reaction end product of the reaction of TCE with FeS is acetylene, and not intermediate dechlorination products such as DCE or VC Thus, biogeochemical transformation has the potential to be a significant degradation pathway for chlorinated ethenes within the biowall without the production of regulated intermediate dechlorination products such as DCE and VC. The process may also occur downgradient of the biowall due to high levels of ferric iron in the native soil.

With time FeS is converted to iron disulfide (FeS_2) minerals (*e.g.*, pyrite) as follows:

(B-11) $FeS\ (s) + S^o \rightarrow FeS_2$

FeS_2 and S^o have not been demonstrated to react with TCE, at least at rates that have significance for the environmental fate and transport of TCE. Therefore, sustaining anaerobic conditions is necessary to prevent oxidation of FeS and to regenerate FeS over time. The rationale for performing the various analyses listed above is provided in the following subsections.

B.4.4.2 Analytical Evaluation for Biogeochemical Transformation

Soil and mulch materials may be analyzed for concentrations of bioavailable iron and manganese, total iron and manganese, and sulfides to evaluate the potential for biogeochemical transformation of CAHs by iron monosulfide to occur. The following describes these analyses, methods for analysis are listed in **Table 6.2**. Additional description of these analyses can be found in AFCEE (2002b), Kennedy and Everett (1999), Wilkin (2003), and Wilkin and Bischoff (2006).

Bioavailable Ferric Iron (Fe^{3+}) is iron that can be utilized as an electron acceptor for microbially mediated iron reduction. Bioavailable ferric iron may be measured with a bioassay test (New Horizons test kit; Evans and Koenigsberg, 2001) that estimates the concentration of bioavailable ferric iron in a soil sample from biogenic ferrous iron (Fe^{2+}) created by the microbial reduction of ferric iron, or by chemical reduction of ferric iron by hydrogen sulfide. Biogenic ferrous iron is measured as the difference in soluble ferrous iron before and after innoculation with iron-reducing bacteria over a 30-day period. Experience has shown that concentrations detected using the bioavailable ferric iron assay test are higher than detected using a weak acid extraction (described below), indicating that the bioassay test may be a better indicator of true bioavailable ferric iron concentrations. Oxidized iron was also calculated by Microseeps, Inc. when conducting the bioavailable iron assay for samples from Altus AFB, Oklahoma (**Appendix F.2**). Any increase in ferric iron over the incubation period may result from oxidation of biogenic ferrous iron. If this occurs, it may be reported as oxidized iron. In this case the sum of the bioavailable ferric iron concentration and the oxidized iron concentration is an approximation of the 'total' concentration of bioavailable ferric iron in the sediment sample.

Weak acid soluble (WAS) iron and manganese represents iron and manganese that are readily accessible for biological or chemical reactions. Microbial/mineral interactions tend to be surface phenomena. Ferric iron or manganese that is reduced under anaerobic conditions is typically on the outer exposed portion of the sediment grain. Alternatively, ferrous iron and manganese can be deposited on sediment surfaces as a coating, as discrete particulates, or may exchange with clay ions. The chemical extraction procedures for iron and manganese speciation normally employ a weak acid solution extractant that dissolves only a small fraction of the total iron and manganese present in the sediment. The goal of the weak acid extraction is to distinguish small quantities of iron and manganese that are readily accessible for biological or chemical reduction from a much larger bulk mass of iron and manganese inherently present in abundance in many sediments. Therefore, WAS iron or manganese is often used as an approximation of bioavailable iron (Fe^{3+}) or bioavailable manganese (Mn^{4+}).

Strong acid soluble (SAS) iron and manganese are iron and manganese extracted by strong acid solution as an estimate of the total amount present in soils. The SAS extracts a greater quantity of native iron and manganese in sediments than the weak acid method. Using

iron as an example, comparing the Fe^{2+} to Fe total ratios between the SAS and WAS results may aid in differentiating zones where Fe^{3+} reduction has occurred (AFCEE, 2002b). Microbial Fe^{3+} reduction often only converts a small amount of the total Fe present in a sediment to Fe^{2+}. In areas where Fe^{3+} reduction has not been enhanced by the presence of relatively abundant organic carbon, the Fe^{2+} to Fe total ratios are approximately the same for both the strong and weak acid extractions for the same sediment. However, in areas where Fe^{3+} reduction has been enhanced, the Fe^{2+} ratios increase for WAS but remain about the same for SAS.

Acid volatile sulfide (AVS) and **chromium extractable sulfide (CES)** are indicative of the amount of reduced metal sulfides in the sediment. In particular, the sulfide in FeS is most susceptible to AVS extraction; therefore, AVS is an approximation of the amount of FeS present in the sediment. CES extraction is an indicator of the fraction of total mineral sulfides extractable by chromium solution. When CES extraction is performed following AVS extraction, then CES is an indication of FeS_2 and S^o remaining in the sediment sample. Because AVS minerals are reactive and do not persist for long periods of time in the environment, the presence of AVS is used as a general indicator of recent sulfate reduction, whereas high CES concentrations relative to AVS concentrations suggests older microbial activity or an increase in the oxidation potential of the groundwater. AVS plus CES yields a total sulfide mineral mass number. Environments rich in AVS relative to CES may indicate recent or on-going biological processes.

B.4.4.3 Laboratory Confirmation of Biogeochemical Transformation

A bench-scale column study was performed by the USEPA National Risk Management Research Laboratory, Ground Water and Ecosystems Restoration Division (NRMRL/GWERD) using biowall material from construction of the SS-17 biowall system at Altus AFB, Oklahoma (Shen and Wilson, 2007). Concentrations of TCE were reduced by over 99% while passing through the columns with a mean residence time of 17 days. After 578 days of operation, approximately 50% of the removal of TCE was attributed to abiotic reactions with FeS that accumulated in the matrix of the column materials, based on calculations of the mass balance of sulfate and sulfide in the influent and effluent for the column studies. Overall first order rate constants for TCE were calculated to range from 0.22 to 0.53 per day. Concentrations of *cis*-1,2-DCE, VC, ethene, ethane, and acetylene could account for only 1% of the TCE removed. However, these compounds are not conservative, and analysis using ^{13}C indicated that the ultimate fate of a significant portion of TCE was abiotic transformation to carbon dioxide.

This column study confirms the potential for biogeochemical transformation of TCE within the Altus AFB biowall system. While it is not practical to attempt a mass balance of sulfate and sulfide at the field scale, measurement of AVS in biowall and native sediments offers another approach to evaluate the potential for biogeochemical transformation (**Appendix D**).

B.5 ANAEROBIC DEGRADATION OF PERCHLORATE

Perchlorate (ClO_4^-) is an anion consisting of a chlorine atom bonded to four oxygen atoms, and is highly soluble and adsorbs poorly to soil (Urbansky, 1998). It is usually produced commercially as the anion of a salt such as ammonium perchlorate (NH_4ClO_4). The physical

and chemical properties of perchlorate and its degradation products are listed in **Table C.1B** in **Appendix C**.

Perchlorate has been shown to degrade anaerobically in the presence of perchlorate-reducing microorganisms (ITRC, 2001 and 2005). Perchlorate is used as an electron acceptor and reduced to chlorate, which is further reduced to chlorite and finally to chloride as follows:

(B-11) ClO_4^-(perchlorate) ➔ ClO_3^-(chlorate) ➔ ClO_2^-(chlorite) ➔ Cl^-(chloride) + O_2

An enzyme (per)chlorate reductase is known to carry out the initial reaction of perchlorate to chlorate and then to chlorite. A second enzyme, chlorite dismutase, subsequently disproportionates chlorite to chloride and oxygen (O_2) (Coates *et al.*, 1999). Many perchlorate-reducing bacteria with these enzymes have been isolated (*e.g.*, see Table 1 in Xu *et al.*, 2003). Wu *et al.* (2001) report that all microorganism capable of reducing perchlorate can similarly reduce chlorate. Therefore, it is unlikely for chlorate to accumulate under anaerobic conditions. While not all microorganisms that can utilize chlorate can also utilize perchlorate, microorganisms capable of using perchlorate as an electron acceptor appear to be ubiquitous in the environment (Xu *et al.*, 2003).

Perchlorate-reducing microorganisms isolated to date are Gram-negative, facultative anaerobes; microorganisms that can grow in either the presence or the absence of oxygen (Xu *et al.*, 2003). While microorganisms capable of reducing both perchlorate and chlorate can tolerate some DO, reduction does not occur in the presence of high concentrations of DO. A suitable source of organic substrate (or molecular hydrogen) is also required to serve as an electron donor. Therefore, microbial degradation of perchlorate in the subsurface is an anaerobic process.

The impact of nitrate on perchlorate reduction is important because nitrate is common in aquifers contaminated with perchlorate. Many perchlorate-reducing microorganisms are also capable of partial or complete denitrification, and the presence of nitrate usually reduces the rate of perchlorate reduction (Xu *et al.*, 2003). However, denitrification and perchlorate reduction are likely to be induced separately and are not exclusive of each other. In the presence of sufficient organic substrate both processes occur. Therefore, the presence of nitrate is not likely to inhibit perchlorate reduction. On the other hand, an inability to stimulate denitrification due to substrate depletion may be a indicator that conditions are not suitable or optimal for perchlorate reduction.

The bulk and reducing equivalents of mulch and compost substrates are usually sufficient to easily overcome the native electron acceptor demand from DO and nitrate, and permeable mulch biowalls and bioreactors are highly suitable for anaerobic bioremediation of perchlorate. Parameters such as DO, nitrate, and TOC are typically monitored to ensure that geochemical conditions are sufficiently reducing for perchlorate reduction.

B.6 ANAEROBIC DEGRADATION OF EXPLOSIVE COMPOUNDS

Potential contaminants in soil and groundwater from the use of explosive munitions include 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, or royal demolition explosive), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX, or high melting point explosive). HMX is a byproduct of the synthesis of RDX and is also used in RDX formulations (McCormick *et al.*, 1981). The physical and chemical properties of TNT, RDX, HMX, and some of their intermediate degradation products are listed in **Table C.1C** in **Appendix C**. The following is a brief description of the anaerobic degradation pathways for TNT, RDX, and HMX that are thought to occur in groundwater. A more thorough summary for degradation of RDX and HMX may be found in GSI (2005).

B.6.1 Anaerobic Degradation of RDX and HMX

Anaerobic biodegradation of RDX has been reported by McCormick *et al.* (1981) in microcosms incubated with anaerobic sewage sludge (**Figure B.7)**. Intermediate products observed during anaerobic degradation included hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX). Concentrations of these products were observed to increase and decrease sequentially as the nitro substituents of RDX were sequentially reduced to the corresponding nitroso- (MNX), dinitroso- (DNX), and trinitroso- (TNX) analogs of RDX. Further degradation also yielded various nitrosamines, dimethylhydrazines, and hydrazines. McCormick *et al.*, (1981) further postulated that the RDX ring cleavage occurred via hydroxylamino intermediates.

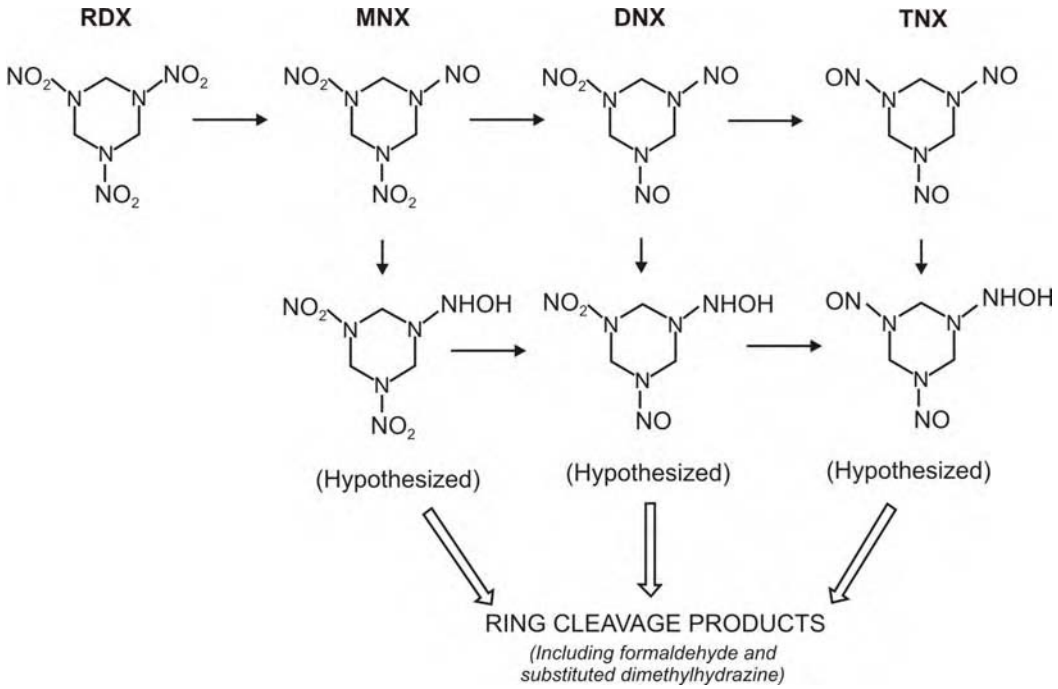


Figure B.7 Biodegradation Pathway for RDX as Postulated by McCormick *et al.* (1981)

Hawari *et al.* (2000) postulated two pathways for anaerobic degradation of RDX in liquid cultures mixed with municipal anaerobic sludge (**Figure B.8**). In addition to the pathway demonstrated by McCormick *et al.*, (1981), Hawari *et al.* (2000) observed a second degradation pathway with the production of the intermediate metabolites methylenedinitramine and bis(hydroxmethyl)nitramine. The two metabolites did not accumulate in the cultures, but were further transformed to innocuous end products such as nitrous oxide and carbon dioxide.

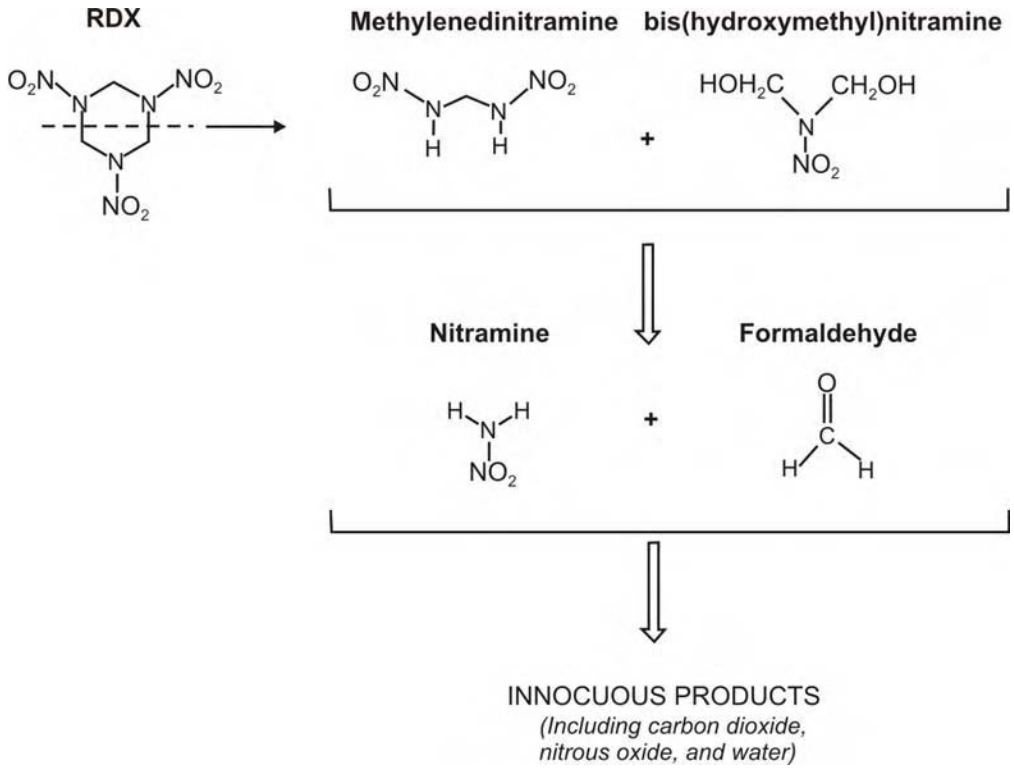


Figure B.8 Biodegradation Pathway for RDX as Postulated by Hawari *et al.* (2000)

More recently, the role of extracellular electron shuttling compounds and ferric iron reduction have been shown to be important microbially-mediated processes in the biodegradation of RDX (Finneran *et al.*, 2007). Finneran *et al.* (2007) were able to demonstrate that electron shuttles mediated biodegradation of RDX more rapidly than previously described microbial or chemical processes, and that biodegradation was more complete with less formation of nitroso or ring-cleavage metabolites. This may be an important process in permeable mulch biowalls or bioreactors where electron shuttles in the form of humic acids may be prevalent.

HMX appears to be more recalcitrant to biodegradation than RDX, as the chemical structure of HMX is reported to be more stable (Hawari *et al.*, 2000). However, Finneran *et al.* (2007) found that the electron shuttling and ferric iron reducing processes for biodegradation of RDX were also applicable to HMX, suggesting that HMX may be amendable to biodegradation in mulch-based biowalls or bioreactors. Because HMX has a

lower solubility limit relative to RDX (**Table C.1C**), it is typically encountered at lower concentrations. In addition, HMX is thought to be less toxic than RDX. The USEPA Region 9 Preliminary Remediation Goals (PRGs) for RDX and HMX in tap water are 0.61 micrograms per liter (µg/L) and 1,800 µg/L, respectively (**Table 2.2**). Therefore, RDX is typically the risk driver where both RDX and HMX are present (GSI, 2005).

B.6.2 Anaerobic Degradation of TNT

Anaerobic biodegradation of TNT has been observed to occur in the laboratory (*e.g.*, Lewis *et al*.,1997; Preuss *et al.,* 1993; and Kahn and Hughes, 1997). In these studies the anaerobic transformation of TNT led to the initial formation of reduced amino derivatives, which were ultimately transformed to 2,4,6-triaminotoluene (TAT). Hawari *et al.* (1998) describe the anaerobic transformation of TNT in an anaerobic sludge culture supplemented with molasses by a two cycle process. In the first cycle, TNT is step-wise reduced to TAT. The second cycle also involved TAT and resulted in the production of azo derivatives, for example 2,2',4,4'-tetraamino-6,6'-azotoluene and 2,2',6,6'-tetraamino-4,4'-azotoluene. The formation and disappearance of TAT were not accompanied by mineralization, suggesting that TAT acted as a dead-end metabolite.

Hwang *et al.* (1998) also observed a near stoichiometric transformation of TNT to TAT in a mixed culture incubated under methanogenic conditions. In this study, TAT was susceptible to further degradation under anaerobic conditions, but its fate was not determined. The addition of ethanol and glucose in the Hwang *et al.* (1998) study enhanced the degradation of TNT, but acetate did not.

In these laboratory studies, the addition of organic substrates stimulated anaerobic transformation of TNT to TAT. Therefore, enhanced *in situ* anaerobic bioremediation of TNT using permeable mulch biowalls and bioreactors appears to hold promise for remediation of TNT in groundwater. As with RDX or HMX, the potential for generation of potentially regulated intermediate degradation products should be evaluated and monitored.

B.6.3 Potential for Enhanced *In Situ* Anaerobic Bioremediation of Explosive Compounds

Microorganisms capable of degrading RDX and TNT include those of the *Clostridia* genus and other microorganisms that display nitroreductase activity (Ederer *et al*., 1993; Regan and Crawford, 1994; Zang and Hughes, 2002; Ahmad and Hughes, 2000 and 2002). These microorganisms are thought to be ubiquitous in the environment. Therefore, RDX and TNT in groundwater are good candidates for remediation using enhanced *in situ* anaerobic bioremediation such as permeable mulch biowalls and bioreactors.

A bench-scale study was performed by GSI (GSI, 2005; Ahmad *et al.*, 2007a) using pine mulch to degrade RDX and HMX as part of a technology demonstration for the Environmental Security Technology Certification Program (ESTCP). Steady-state column flow-through tests were run at average seepage velocity for the field demonstration site at the Army Pueblo Chemical Depot (PCD), Colorado (GSI, 2005). The columns were packed with a mixture of pine mulch and pea gravel at a ratio of 70 percent by volume mulch to 30 percent by volume pea gravel. Results of the column study included 1) complete removal of RDX and HMX at influent concentrations of 90 µg/L and 8 µg/L, respectively; 2) a pseudo first-order steady-state rate constant for RDX of 0.20 to 0.27 per hour; 3) low accumulation of

RDX intermediates in the column effluent at less than 2 percent of the influent RDX mass; and 4) no apparent binding (sorption) of RDX to the column fill material. Based on these results, a field demonstration of a permeable mulch biowall is underway at the PCD, Colorado. Design of the biowall was based in large part on results of the bench-scale study.

Bench-scale studies using other organic substrates to stimulate anaerobic degradation of RDX have also been conducted. Jerger *et al.* (2001) conducted microcosm studies using molasses as an organic substrate to anaerobically degrade RDX and HMX in groundwater at the Department of Energy (DOE) Pantex facility in West Texas. Concentrations of RDX of approximately 5,000 µg/L were degraded to less than 5.0 µg/L within 30 days. The appearance and disappearance of MNX, DNX, and TNX indicated these compounds were intermediate degradation products that were formed but did not accumulate. Degradation of HMX was also observed. Wani and Davis (2003), Wani *et al.* (2002), Zang and Hughes (2002), and Heaston *et al.* (2001) describe additional bench-scale studies where RDX was degraded by anaerobic processes stimulated by addition of various organic substrates.

APPENDIX C

REFERENCE TABLES

Table C.1A Characteristics of Chlorinated Aliphatic Hydrocarbons and Dechlorination End Products

Compound	Molecular Formula	Molecular Weight (g/mol)[a/]	Density (g/mL @ approx. 20 to 25°C)[b/]	Henry's Law Constant (atm-m^3/mol)[e/]	Solubility (mg/L @ approx. 20 to 25°C)[c/]	Vapor Pressure (mm Hg @ 20 °C)[d/]	Octanol/Water Partition Coefficient (log Kow)[f/]	Octanol/Carbon Partition Coefficient (log Koc)[g/]
Chloroethenes								
Tetrachloroethene (PCE)	C_2Cl_4	165.8 (1)	1.62 (1)	0.0132 (2)	150 (3)	14.0 (3)	2.53 (4)	2.42 (5)
Trichloroethene (TCE)	C_2HCl_3	131.4 (1)	1.46 (1)	0.0072 (2)	1,100 (3)	60.0 (3)	2.42 (4)	2.03 (5)
cis-1,2- Dichloroethene (*cis*-1,2-DCE)	$C_2H_2Cl_2$	96.94 (1)	1.28 (1)	0.0030 (2)	3,500 (3)	200 (6)	1.86 (10)	1.65 (7)
trans-1,2- Dichloroethene (*trans*-1,2-DCE)	$C_2H_2Cl_2$	96.94 (1)	1.26 (1)	0.0073 (2)	6,300 (4)	340 (6)	2.09 (10)	1.77 (5)
1,1-Dichloroethene (1,1-DCE)	$C_2H_2Cl_2$	96.94 (1)	1.22 (1)	0.021 (2)	2,250 (5)	500 (3)	2.13 (4)	1.81 (5)
Vinyl Chloride (VC)	C_2H_3Cl	62.51 (1)	Gas	0.218 (2)	1,100 (3)	2,660 (3)	0.60 (4)	1.23 (5)
Ethene	C_2H_4	28.05 (1)	Gas	8.60 (7)	131 (7)	30,800 (7)	1.13 (8)	2.48 (7)
Acetylene	C_2H_2	26.04 (10)	Gas	0.0217 (10)	1,200 (10)	40,400 (10)	0.37 (10)	NA
Chloroethanes								
1,1,1,2-Tetrachloroethane (1,1,1,2-TCA)	$C_2H_2Cl_4$	167.85 (1)	1.553 (10)	0.0025 (10)	1,070 (10)	12 (10)	2.93 (10)	NA
1,1,2,2-Tetrachloroethane (1,1,2,2-TCA)	$C_2H_2Cl_4$	167.85 (1)	1.595 (1)	0.00038 (4)	2,962 (6)	5.0 (3)	2.56 (4)	2.07 (4)
1,1,1-Trichloroethane (1,1,1-TCA)	$C_2H_3Cl_3$	133.4 (1)	1.34 (1)	0.0133 (2)	4,400 (3)	100 (3)	2.47 (4)	2.02 (5)
1,1,2-Trichloroethane (1,1,2-TCA)	$C_2H_3Cl_3$	133.4 (1)	1.44 (1)	0.0012 (7)	4,500 (3)	19 (3)	2.18 (4)	1.75 (5)
1,1-Dichloroethane (1,1-DCA)	$C_2H_4Cl_2$	98.96 (1)	1.18 (1)	0.0043 (2)	5,500 (3)	180 (3)	1.78 (4)	1.48 (5)
1,2-Dichloroethane (1,2-DCA)	$C_2H_4Cl_2$	98.96 (1)	1.24 (1)	0.00098 (6)	8,690 (3)	61 (3)	1.48 (4)	1.28 (5)
Chloroethane (CA)	C_2H_5Cl	64.51 (1)	Gas	0.0094 (2)	5,740 (3)	1,010 (3)	1.43 (4)	1.42 (7)
Ethane	C_2H_6	30.07 (1)	Gas	19.2 (7)	60.4 (3)	29,300 (3)	1.81 (8)	2.66 (7)

(continued)

Table C.1A Characteristics of Chlorinated Aliphatic Hydrocarbons and Dechlorination End Products (concluded)

Compound	Molecular Formula	Molecular Weight (g/mol)[a/]	Density (g/mL @ approx. 20 to 25°C)[b/]	Henry's Law Constant (atm-m^3/mol)[e/]	Solubility (mg/L @ approx. 20 to 25°C)[c/]	Vapor Pressure (mm Hg @ 20°C)[d/]	Octanol/Water Partition Coefficient (log Kow)[f/]	Octanol/Carbon Partition Coefficient (log Koc)[g/]
Chloromethanes								
Tetrachloromethane/ Carbon Tetrachloride (CT)	CCl_4	153.8 (1)	1.58 (1)	0.0232 (4)	786 (4)	90 (3)	2.73 (4)	2.62 (4)
Trichloromethane/ Chloroform (CF)	$CHCl_3$	119.4 (1)	1.48 (1)	0.00367 (2)	8,000 (3)	160 (3)	3.98 (4)	1.45 (9)
Dichloromethane (DCM) (or methylene chloride)	CH_2Cl_2	84.93 (1)	1.33 (1)	0.00244 (4)	19,400 (4)	380 (4)	1.25 (4)	1.44 (4)
Chloromethane (CM)	CH_3Cl_1	50.48 (4)	Gas	0.00882 (2)	6,500 (4)	4,310 (4)	0.91 (4)	1.40 (4)
Methane	CH_4	16.04 (1)	Gas	18.3 (7)	24 (3)	20,800 (7)	1.09 (8)	2.88 (7)

Table C.1B Characteristics of Perchlorate and Degradation Products

Compound	Molecular Formula	Molecular Weight (g/mol)[a/]	Density (g/mL @ approx. 20 to 25°C)[b/]	Henry's Law Constant (atm-m^3/mol)[e/]	Solubility (mg/L @ approx. 20 to 25°C)[c/]	Vapor Pressure (mm Hg @ 20°C)[d/]	Octanol/Water Partition Coefficient (log Kow)[f/]	Octanol/Carbon Partition Coefficient (log Koc)[g/]
Ammonium Perchlorate	NH_4ClO_4	117.49 (10)	1.95 (16)	NA	200,000 (10)	2.81E-11 @ 25ºC (10)	-5.84 (10)	Very low
Sodium Perchlorate	$NaClO_4$	122.44 (10)	2.52 (16)	NA	2,100,000 (10)	2.07E-16 @ 25ºC (10)	-7.18 (10)	Very Low
Potassium Perchlorate	$KClO_4$	138.55 (10)	2.53 (16)	NA	15,000 (10)	2.07E-16 @ 25ºC (10)	-7.18 (10)	Very Low
Perchloric Acid (perchlorate)	$HClO_4$	100.46 (13)	1.66 (13)	NA	Miscible in cold water	NA	-4.63 (13)	Very Low
Chloric Acid (chlorate)	$HClO_3^-$	84.46 (13)	NA	NA	NA	NA	NA	Very Low
Chlorous Acid (chlorite)	$HClO_2^-$	68.46 (13)	NA	NA	NA	NA	NA	Very Low
Hydrochloric Acid	HCl^-	36.46 (13)	0.909 (13)	NA	62,000 (13)	NA	NA	Very Low
Chloride	Cl^-	35.45 (10)	NA	0.00773 (10)	42,400 (10)	4.16E-08 @ 25ºC (10)	0.54 (10)	Very Low

Table C.1C Characteristics of Energetics and Degradation Products

Compound	Molecular Formula	Molecular Weight (g/mol)[a/]	Density (g/mL @ approx. 20 to 25°C)[b/]	Henry's Law Constant (atm-m^3/mol)[e/]	Solubility (mg/L @ approx. 20 to 25°C)[c/]	Vapor Pressure (mm Hg @ 20°C)[d/]	Octanol/Water Partition Coefficient (log Kow)[f/]	Octanol/Carbon Partition Coefficient (log Koc)[g/]
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	$C_3H_6N_6O_6$	222.15 (11)	1.830 (11)	1.96E-11 (11)	60 (11)	4.03E-09 (11)	0.87 (15)	0.13 (7)
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	$C_4H_8N_8O_8$	296.20 (11)	1.900 (11)	2.60E-15 (11)	5 (11)	3.33E-14 (11)	0.82 (15)	0.02 (7)
2,4,6-Trinitrotoluene (TNT)	$C_7H_5N_3O_6$	227.15 (11)	1.650 (11)	1.10E-08 (11)	150 (11)	1.50E-04 (11)	1.60 (15)	0.19 (7)
2,4-Dinitrotoluene	$C_7H_6N_2O_4$	182.15 (11)	1.521 (11)	1.86E-07 (11)	280 (11)	2.17E-04 (11)	1.98 (15)	0.19 (7)
2,6-Dinitrotolune	$C_7H_6N_2O_4$	182.15 (11)	1.538 (11)	4.86E-07 (11)	206 (11)	5.67E-04 (11)	2.10 (15)	0.19 (7)
2-Nitrotoluene	$C_7H_7NO_2$	137.14 (10)	1.163 (13)	1.25E-05 (10)	650 @ 30ºC (10)	0.188 @ 25ºC (10)	2.30 (10)	2.50 (15)
3-Nitrotoluene	$C_7H_7NO_2$	137.14 (10)	1.157 (13)	9.30E-06 (10)	500 @ 30ºC (10)	0.205 @ 25ºC (10)	2.45 (10)	2.49 (15)
4-Nitrotoluene	$C_7H_7NO_2$	137.14 (10)	1.392 (13)	5.63E-06 (10)	442 @ 30ºC (10)	0.0157 @ 25ºC (10)	2.37 (10)	2.49 (15)
1,3,5-Trinitrobenzene	$C_6H_3N_3O_6$	213.11 (10)	1.4775 @ 152ºC (14)	1.30E-15 (10)	278 @ 15ºC (10)	6.44E-06 @ 25ºC (10)	1.18 (10)	3.03 (15)
1,3-Dinitrobenzene	$C_6H_4N_2O_4$	168.11 (10)	1.368 (13)	4.9E-08 (10)	533 (10)	9.00E-04 @ 25ºC (10)	1.49 (10)	2.34 (15)
Nitrobenzene	$C_6H_5NO_2$	123.11 (12)	1.200 (12)	1.53E-05 (12)	1,900 (12)	0.150 @ 25ºC (12)	1.85 (15)	0.18 (7)
4-Amino-2,6-Dinitrotoluene	$C_7H_7N_3O_4$	197.15 (10)	1.57 (16)	3.27E-11 (10)	1,220 (10)	1.07E-05 @ 25ºC (10)	1.84 (10)	2.00 (15)
Tetryl	$C_7H_5N_5O_8$	287.17 (11)	1.730 (11)	2.96E-11 (11)	80 (11)	5.69E-09 (11)	1.64 (16)	0.17 (7)
Nitroglycerin	$C_3H_5N_3O_9$	227.09 (10)	1.5918 (16)	8.66E-08 (10)	1,380 (10)	4.00E-04 @ 25ºC (10)	1.62 (10)	2.12 (15)

[a/] g/mol = grams per mole.

[b/] g/ml = grams per milliliter; °C = degrees Celsius.

[c/] mg/L = milligrams per liter.

[d/] mm Hg = vapor pressure measured as millimeters of mercury.

[e/] atm-m^3/mol = atmospheres-cubic meter per mole.

[f/] log Kow = log of octanol/water partition coefficient (dissolution coefficient).

[g/] log Koc = log of octanol/carbon coefficient (soil sorption coefficient).

(continued)

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TABLE C.2
POTENTIAL COTTON DEFOLIANTS

Defoliant	Active Ingredients	Product Persistence/Degradability
GINSTAR EC	Thidiazuron: N-phenyl-N'-1,2,3-thidiazol-5-ylurea (12%); Diuron: 3-(3,4-dichlorophenyl)-1,1-dimethylurea (6%); Contains 1 lb Thidiazuron per gallon and 0.5 lb Diuron per US gallon.	5 months [a/]
FINISH 6	Ethephon (2-chloroethyl) phosphonic acid (51.4%); Cyclanilide 1-(2,4-dichlorophenylaminocarbonyl) – cyclopropane carboxylic acid (6.4%); Contains 6.0 lb ethephon per gallon and 0.75 lb cyclanilide per gallon.	4 months [a/]
FINISH 6 PRO	Ethephon (2-chloroethyl) phosphonic acid (52.6%); Cyclanilide 1-(2,4-dichlorophenylaminocarbonyl) – cyclopropane carboxylic acid (3.3%); Contains 6.0 lb ethephon per gallon and 0.375 lb cyclanilide per gallon.	4 months [a/]
FOLEX 6	S, S, S-Tributyl phosphorotrithioate (70.5%); Contains 6 lb S, S, S-Tributyl phosphorotrithioate per gallon; Contains petroleum distillates.	24 days [b/]
GRAMOXONE EXTRA	Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) (37%); Contains 2.5 lb paraquat cation per gallon. Contains stench (odor) and emetic.	70 days [a/]
GRAMOXONE MAX	Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) (43.8%); Contains 3.0 lb paraquat cation per gallon as 4.143 lb salt per gallon. Contains stench (odor) and emetic.	70 days [a/]
GRAMOXONE SUPER TRES	Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) (43.8%); Contains 3.0 lbs paraquat cation per gallon as 4.143 lb salt per gallon. Contains stench (odor) and emetic.	60 days [a/]
PREP	Ethephon (2-Chloroethyl) phosphonic acid (55.4%); Contains 6 lb ethephon per gallon.	30 days [a/]
DEF 6	S,S,S-Tributyl phosphorotrithioate (70.5%); Contains 6 lb tribufos: S,S,S-Tributyl phosphorotrithioate per gallon; Contains petroleum distillates.	24 days [b/]

Sources: Humphreys Co-Op in Altus Oklahoma and www.greenbook.net.
Notes: a/ - Product Persistence/Degradability is determined based on the maximum period before planting a crop after application.
b/ - Product Persistence/Degradability is determined based on the half-life in soil.

APPENDIX D

EVALUATING AND STIMULATING BIOGEOCHEMICAL TRANSFORMATION OF CHLORINATED SOLVENTS BY REACTION WITH IRON MONOSULFIDE

APPENDIX D

EVALUATING AND STIMULATING BIOGEOCHEMICAL TRANSFORMATION OF CHLORINATED SOLVENTS BY REACTION WITH IRON MONOSULFIDE

D.1 INTRODUCTION

This appendix provides example calculations to evaluate the occurrence and stimulation of biogeochemical transformation of chlorinated aliphatic hydrocarbons (CAHs, or chlorinated solvents) by reaction with iron monosulfide minerals (FeS). Biogeochemical transformation can be a significant degradation process using mulch and compost substrates in environments where sources of sulfate and iron are present (*e.g.*, Shen and Wilson, 2007; Kennedy and Everett, 2003). Attempts have been made to engineer the process by addition of iron- or sulfate-bearing amendments to biowall backfill materials (*e.g.*, Parsons 2006c and 2007a).

There is still a limited understanding of the processes involved, and a significant amount of research and development is needed before biogeochemical transformation of CAHs can be engineered with confidence (Air Force Center for Engineering and the Environment [AFCEE] *et al.*, 2008). Specifically, the hypothesized degradation mechanisms or pathways need to be confirmed, mechanisms of active mineral formation need to be defined, and the interplay between abiotic and biological degradation processes requires more investigation. This interplay is important as the production and regeneration of high surface area mineral phases with a high degree of reactivity is dependent on both biological activity and the availability of iron and sulfate in the reaction zone.

In the meantime, this appendix describes some simple approaches used to evaluate the potential for stimulating biogeochemical transformation of CAHs by reaction with FeS, including the addition of iron or sulfate amendments where they may be limiting factors. This discussion is based on degradation of trichloroethene (TCE), primarily because biogeochemical transformation of this compound has been researched to a greater degree than most other CAHs (*e.g.*, Butler and Hayes, 1999 and 2001; Lee and Batchelor, 2000, 2002a and 2002b; Weerasooriya and Dharmasena, 2001; Liang *et al.*, 2006; Kennedy *et al.*, 2006; Shen and Wilson, 2007). The abiotic transformation of TCE by reaction with iron sulfides is described in **Appendix B.4.4**, and sufficient information is retained here to provide the necessary stoichiometry for the calculations described.

D.2 TECHNICAL APPROACH

The potential for accumulation of FeS may be estimated given the concentrations of sulfate in groundwater and iron in the backfill material. For existing biowalls or bioreactors, sampling techniques (**Section 6.3**) and analytical methods (**Section 6.5**) are available to measure the acid volatile sulfide (AVS) content of the material as an estimate of the amount of FeS present. Site-specific groundwater hydraulics and biowall/bioreactor dimensions and backfill properties can be used to estimate the volumetric flow rate and residence time of TCE in the biowall or bioreactor. Given the initial (upgradient) concentration of TCE and the mass of FeS present, an estimate of the degree to which TCE may be degraded in the reaction zone can be made assuming a conservative rate of abiotic degradation (*e.g.*, Shen and Wilson, 2007). These calculations can also be used to justify either increasing the amount of sulfate and/or iron to

generate higher concentrations of FeS, or to increase the biowall or bioreactor dimensions in order to increase residence/contact time.

D.2.1 Potential for Accumulation of FeS

Stoichiometric relationships are used to estimate the potential amount of FeS that may accumulate in the biowall/bioreactor over time, using the concentration of sulfate in groundwater and the mass of iron in the backfill material. It is assumed that sufficient organic carbon is available to sustain both sulfate reduction and iron reduction, which is reasonable based on the initial mass of organic material typically emplaced in a biowall or bioreactor. Performance monitoring indicates that anaerobic conditions (*i.e.*, sulfate reduction) may be sustained over periods of 3 to 5 years (or more) without additional substrate amendment (*e.g.*, see **Appendix F.2**). However, a decrease in sulfate reduction may indicate that the amount of organic substrate is no longer sufficient to sustain the process.

Using a stoichiometric approach, it becomes apparent whether dissolved sulfate or iron in the biowall matrix is a limiting factor in the generation of FeS. The amount of sulfate and/or iron that may be added to the biowall backfill material to potentially produce a greater mass of FeS can be estimated using the same stoichiometric relationships. From a practical standpoint it is necessary to a have a greater amount of sulfate and iron than theoretically required. Not all the sulfate or iron may be reduced to form FeS, and the residence or contact time for TCE with FeS may be a limiting factor.

D.2.2 Potential for Degradation of TCE Based on Exposure to FeS

Another set of calculations may be performed to evaluate the potential to abiotically degrade TCE to meet the performance criterion, based on the rate at which TCE is exposed to FeS and using degradation rates listed in the literature. This appendix uses degradation rates calculated by Shen and Wilson (2007) for removal of TCE by exposure to FeS in column studies using biowall materials and native groundwater from Altus Air Force Base (AFB), Oklahoma. The normalized rate of abiotic degradation is assumed to be a first-order reaction and is expressed in terms of the rate per day (d^{-1}) when exposed to 1.0 mole of FeS in contact with 1.0 liter of pore water (M^{-1}). Rates associated with degradation of TCE by reaction with FeS calculated by Shen and Wilson (2007) varied from 0.53 to 2.3 $d^{-1}M^{-1}$.

D.2.3 Amending the Reactive Matrix with Iron or Sulfate

Amendments that have been used for adding sulfate include anhydrite (calcium sulfate), gypsum (anhydrous calcium sulfate), and Epsom salts (anhydrous magnesium sulfate or hexahydrite). Agricultural products such as gypsum fertilizer pellets are available that have varying concentrations of sulfate. The most common approach for increasing the concentration of iron is to screen local sources of sand for the concentration of total iron, and to select the material with the highest concentration. Magnetite ore has been added on an experimental basis to portions of biowalls at Altus AFB, Oklahoma and Ellsworth AFB, South Dakota. Other potential iron amendments might include iron rich minerals such as hematite or limonite, ferrous iron sulfate (a common soil amendment used to raise pH), or ferrous iron chloride.

D.3 CALCULATIONS

Evaluating biogeochemical transformation of CAHs by reaction with FeS minerals requires determination of the following:

1) The volumetric flow rate (discharge) and residence time of groundwater moving through a biowall or bioreactor (**Section D.3.1**);

2) The influx of dissolved CAHs and sulfate mass into a biowall or bioreactor (**Section D.3.2**);

3) The stoichiometry of sulfate and iron reduction to form FeS (**Section D.3.3**);

4) The mass of FeS that may be produced from native dissolved sulfate and iron in the biowall backfill based on stoichiometry, and the potential to degrade CAHs (**Section D.3.4**);

5) The mass of FeS required to degrade the mass of CAHs based on exposure to FeS and rates published in the literature (**Section D.3.5**); and

6) Whether sulfate and/or iron amendments should be applied to optimize the production of FeS (**Section D.3.6**).

The following subsections describe the stoichiometry and rate calculations that are useful for determining the above factors. A biowall configuration designed to treat TCE is used for illustration (the OU-1 biowall at Altus AFB), but the same principles apply to other CAHs and to other configurations such as recirculating bioreactors.

D.3.1 Calculating the Volumetric Flow Rate and Residence Time of Groundwater in a Biowall

Calculating the rate of groundwater flow through a biowall is described in **Section 3.5.2** using a simplistic approach following Darcy's Law. Darcy's Law states that the volumetric flow rate (Q) through a pipe filled with sand can be calculated as follows:

$$Q = - KA(dh/dl) \qquad \text{(D-1)}$$

where

The negative sign indicates that flow is in the direction of decreasing hydraulic head;

K = proportionality constant (length divided by time [L/T]);

A = the cross sectional area of the pipe (L^2); and

dh/dl = the horizontal hydraulic gradient (unitless).

Darcy's Law may also be applied to flow through a porous medium where the proportionality constant is the hydraulic conductivity. As an example, the OU-1 biowall described in **Appendix F.2** is approximately 455 feet long with an average saturated thickness (vertical dimension) of approximately 18 feet. Given an average site-wide hydraulic gradient of -0.003 foot per foot (ft/ft) and an average site-wide hydraulic conductivity of 8.7 feet per day (ft/day), the volumetric flow rate (Q) across the biowall can be estimated as follows:

$Q = - KA(dh/dl)$

Q = -(8.7 ft/day) (455 ft) (18 ft) (-0.003 ft/ft) = 214 cubic feet per day (ft^3/day), or

Q = (214 ft^3/day) (28.32 liters per cubic feet) = 6,060 liters per day (L/day)

Equation D-1 can be solved to yield the Darcy velocity or specific discharge. As defined, the specific discharge (q) is a volumetric flow rate per unit surface area of a porous medium:

$$q = Q/A = - K(dh/dl) \quad \text{(D-2)}$$

This equation is useful because the water balance (flow in versus flow out) across a biowall of limited thickness (less than a few feet) can be assumed to be approximately equal to the volumetric flow of water through the aquifer. For the OU-1 biowall example:

$q = Q/A = - K(dh/dl)$

q = -(8.7 ft/day) (-0.003 ft/ft) = 0.026 ft/day, or

q = (0.026 ft/day)(30.48 centimeters per foot) = 0.79 centimeters per day (cm/day)

Because water only moves through the interconnected pore openings of an aquifer, Darcy's q is a superficial or apparent velocity. That is, q represents the velocity at which water would flow if the aquifer were an open conduit, but does not account for dispersion that causes water to flow through different pore spaces at different rates along individual flow paths that vary in length**.** The velocity of water through the aquifer pore spaces is termed the average linear velocity or seepage velocity where:

$$v = - K(dh/dl) / n_e \quad \text{(D-3)}$$

where

v = average linear or seepage velocity (L/T); and

n_e = effective porosity of the aquifer matrix (unitless)

To calculate the average linear groundwater velocity across a biowall, one must know or estimate the effective porosity of the mulch mixture. Ahmad *et al*. (2007b) evaluated the effective water-filled porosity of biowall materials, and reported that a conservative approximation of the effective porosity of biowall backfill material is 40 percent where the mulch fraction ranges from 40 to 60 percent by volume. Shen and Wilson (2007) report a water-filled porosity of 42 percent and an effective porosity of 25 percent for columns constructed with material from the SS-17 biowall at Altus AFB at a ratio of 50 percent shredded tree mulch, 10 percent cotton gin trash, and 40 percent sand by volume.

For the OU-1 biowall example in **Appendix F.2,** the seepage velocity of groundwater in the native formation was estimated to be 0.174 ft/day based on a hydraulic gradient of -0.003 ft/ft, an average aquifer hydraulic conductivity of 8.7 ft/day, and an effective porosity of 15 percent. Assuming that the specific discharge (q) is the same across the biowall as it is in the aquifer, then the seepage velocity across the biowall can be estimated as 0.10 ft/day using an effective

porosity of 25 percent and Equation D-3. With a biowall width of 1.5 feet, the mean residence time of groundwater within the OU-1 biowall is estimated to be 15 days.

Groundwater residence time is a conservative estimate of contaminant residence time because it does not account for the effects of sorption and retardation of organic compounds. For example, Shen and Wilson (2007) estimated a retardation factor of 12 for TCE in column studies using a mulch mixture from the SS-17 biowall at Altus AFB, Oklahoma. Therefore, the mean residence time of TCE in the OU-1 biowall may be as high as 180 days.

D.3.2 Calculating the Influx of Mass Into a Biowall

A simple method to calculate the influx of mass of CAHs or dissolved electron acceptors (*e.g.*, sulfate) into a biowall is to multiple the volumetric flow rate (Q) by an average or mean concentration of each constituent. For example, given a Q of 6,060 L/day and an average initial TCE concentration (C_o) of 2,000 micrograms per liter (µg/L), the mass influx of TCE into the OU-1 biowall can be calculated as follows:

Mass of TCE per day = Q (L/day) x C_o (µg/L) (D-4)

= (6,060 L/day) (2,000 µg/L) = 12,120,000 µg/day

= 12.12 grams per day (g/day)

Thus, approximately 12.12 grams of TCE enters the biowall per day. Likewise, the mass discharge of TCE exiting the biowall could be estimated using an average or mean concentration of TCE measured on the downgradient side of the biowall. For an average concentration of 2,000 milligrams per liter (mg/L) sulfate, the mass influx of sulfate into the biowall can similarly be calculated to be approximately 12.12 kilograms per day (kg/day).

D.3.3 Stoichiometry of Biogeochemical Transformation of TCE by FeS

Analysis of native soil and biowall backfill material may be performed to evaluate the presence of iron and sulfides in aquifer sediments and within the biowall reactive media. The following discussion describes the stoichiometry of producing FeS using common oxidation-reduction reactions (from **Appendix B.4.4**).

The biological reduction of sulfate (SO_4^{2-}) coupled with the oxidation of organic material by sulfate-reducing bacteria produces hydrogen sulfide (H_2S or HS^-), for example in the following reactions (from AFCEE, 2002b):

$$CH_3COOH\ (organic) + SO_4^{2-} + 2H_2O \rightarrow 2CO_2 + H_2S + 2H_2O + 2OH^- \quad \text{(D-5)}$$

$$2CH_2O\ (organic) + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad \text{(D-6)}$$

Using these reactions, the reduction of one mole of sulfate produces one mole of hydrogen sulfide.

Ferric iron (Fe^{3+}) in the subsurface soil or biowall material may be reduced to ferrous iron (Fe^{2+}) by either biological or chemical processes. The biological reduction of ferric iron to ferrous iron may proceed as follows (from AFCEE, 2002b):

$$CH_2O\ (organic) + 4Fe^{3+}(s) \rightarrow 4Fe^{2+}(aq) + HCO_3^- + 5H^+ \quad \text{(D-7)}$$

Most ferrous iron will precipitate in mineral form, for example with sulfides to form FeS or iron disulfide (FeS_2), with carbonate to form siderite ($FeCO_3$), or with other iron oxides/hydroxides to form magnetite (Fe_3O_4).

Iron oxide or iron hydroxide minerals provide a strong chemical sink for H_2S, forming various iron sulfide minerals. Hydrogen sulfide may chemically reduce Fe^{3+} present in iron oxides or iron hydroxides to form FeS, for example with goethite according to the following reaction (from AFCEE, 2002b):

$$2FeOOH\ (s)\ (goethite) + 3H_2S\ (aq) \rightarrow 2FeS(s) + S^o + 4H_2O \quad \text{(D-8)}$$

Using this equation, two moles of goethite (iron hydroxide) reduced by three moles of hydrogen sulfide produces two moles of FeS. Precipitated iron sulfide mineral forms include amorphous iron sulfide (FeS), mackinawite (FeS), pyrrhotite (FeS), greigite (Fe_3S_4), and others (AFCEE, 2002b).

FeS minerals, which exist in a reduced state, may react rapidly with oxidized compounds such as TCE to form acetylene (Butler and Hayes, 1999). The suggested chemical expression for TCE dechlorination via FeS oxidation is (from Kennedy and Everett, 2003):

$$4\ FeS + 9\ C_2HCl_3\ (TCE) + 28\ H_2O \rightarrow \quad \text{(D-9)}$$
$$4\ Fe(OH)_3 + 4\ SO_4^{2-} + 9\ C_2H_2\ (acetylene) + 27\ Cl^- + 35\ H^+$$

Using this equation, four moles of FeS is sufficient to degrade nine moles of TCE. Based on the molar mass (**Table D-1**) of FeS (87.91) and TCE (131.39), it takes approximately 0.30 milligram of FeS to degrade 1.0 milligram TCE. However, as noted in **Appendix B.4.4**, the degree to which FeS is actually oxidized by reduction of TCE is uncertain (Kennedy and Everett, 2003). Therefore, an excess of FeS will be required under field conditions to optimize contact with TCE and to facilitate other oxidation reactions with FeS.

Table D-1 Molecular or Elemental Mass of Compounds and Elements Involved in Biogeochemical Transformation of TCE by FeS

Compound	Molecular Formula/Element	Molar/Elemental Mass (g/L)
Trichloroethene (TCE)	C_2HCl_3	131.39
Iron Monosulfide	FeS	87.91
Iron Disulfide	FeS_2	119.97
Elemental Sulfur or Sulfide	S° or S^{2-}	32.06
Ferric or Ferrous Iron	Fe^{3+} or Fe^{2+}	55.85
Sulfate	SO_4^{-2}	96.06
Hydrogen Sulfide	H_2S or HS^-	34.08 or 33.07

Note: g/L = grams per liter.

In summary, the following mass relationships can be assumed based on the stoichiometry presented above:

- Based on the molar to molar production of H_2S from SO_4^{2-} (Equation D-5 or D-6), the molar mass of SO_4^{2-} (96.06), the molar mass of FeS (87.91), and Equation D-8, *it takes approximately 1.64 milligrams of SO_4^{2-} to produce 1.0 milligram of FeS.*

- Based on the elemental mass of Fe^{3+} (55.85) and molar mass of FeS (87.91), and Equation D-8, *it takes approximately 0.64 milligram of Fe^{3+} to produce 1.0 milligram of FeS* by chemical reduction of ferric iron (goethite).

- Based on the molar mass of FeS (87.91) and TCE (131.39), and Equation D-9, *it takes approximately 0.30 milligram of FeS to degrade 1.0 milligram TCE.*

These mass relationships may be used to evaluate the potential to generate FeS based on site-specific conditions and the potential to degrade TCE by reaction with FeS.

D.3.4 Potential for Accumulation of FeS from Reduction of Native Sulfate and Iron

Biogeochemical transformation of TCE by FeS may occur when sufficient organic carbon, ferric iron, and sulfate are present. For the Altus OU-1 biowall (**Appendix F.2**), organic carbon is present in the form of mulch and compost, ferric iron is present both in the river sand used for biowall backfill and in the native sediments, and elevated concentrations of sulfate occur naturally in groundwater as a result of dissolution of gypsum and anhydrite from the aquifer matrix.

D.3.4.1 Sulfate Demand

An example calculation of the mass influx of sulfate into the OU-1 biowall was shown in **Section D.3.2** above; this flux was determined to be approximately 12.12 kg/day of sulfate assuming a background concentration of 2,000 mg/L sulfate in groundwater. Shen and Wilson (2007) estimated that the sulfate demand exerted by the mulch mixture in their columns was approximately 25 mg/L per day. This rate of consumption of sulfate can be compared to the availability of sulfate in groundwater at the OU-1 biowall, where a similar mulch backfill material was used.

The dimensions of the saturated portion of the OU-1 biowall are approximately 455 feet long by 18 feet deep by 1.5 feet wide, with a volume of 12,285 cubic feet; or approximately 347,863 liters. Given a water filled porosity of 40 percent, then the saturated portion of the biowall contains approximately 139,145 liters of water. At a sulfate demand of 25 mg/L per day, the demand for sulfate is approximately 3.48 kg/day. Therefore, with an influx of 12.12 kg/day the supply of sulfate into the biowall is sufficient to meet the estimated sulfate demand.

D.3.4.2 Available Iron

Approximately 265 cubic yards (202 cubic meters) of sand was procured for the OU-1 biowall, assuming the biowall would be filled to a total vertical height of 24 feet. Given the saturated portion is 18 feet, about 200 cubic yards (153 cubic meters) of sand are present within the saturated portion of the biowall trench. Given that the sand has a bulk density of 1.66 grams per cubic centimeter (g/cm^3), or 1,660 kilograms per cubic meter (kg/m^3), the total mass of sand

within the saturated portion of the biowall is 254,000 kilograms. The iron content of the sand fraction collected from above the water table was measured by Kennedy and Everett (2003) to be 12,800 milligrams per kilogram (mg/kg), or 1.28 percent. Therefore, the initial mass of iron in the biowall matrix (saturated portion) can be estimated to be approximately 3,250 kilograms.

This bulk mass of iron does not indicate the rate at which it may be reduced to form FeS, or the extent to which it will be used in other reactions. Some iron may be reduced to soluble ferrous iron that may migrate out of the biowall reaction zone with groundwater flow. In addition, not all the iron in the sand matrix is in the ferric state (many iron minerals are comprised of mixed valence states of iron), and not all the iron may be in a form that can be reduced by chemical or biological processes.

However, assuming that 1) all the iron can be reduced, 2) only the reactions in Equations D-5 through D-9 occur, and 3) the reactions are instantaneous, a minimum mass of ferric iron can be calculated to balance the sulfate demand. As stated earlier, it takes 1.64 milligrams of SO_4^{2-} and 0.64 milligram of Fe^{3+} to produce 1.0 milligram of FeS. Given a sulfate demand of 3.48 kg/day, then the amount of iron needed to react with the H_2S formed by sulfate reduction to generate FeS is approximately:

FeS from sulfate = (3.48 kg/day SO_4^{2-}) / (1.64 mg SO_4^{2-} per 1.0 mg FeS)

= 2.12 kg/day FeS

Fe^{3+} to produce FeS = (2.12 kg/day FeS) (0.64 mg Fe^{3+} per 1.0 mg FeS)

= 1.36 kg/day Fe^{3+}

Therefore, approximately 1.36 kg/day of Fe^{3+} is needed to balance the estimated sulfate demand of 3.48 kg/day, and this results in the production of 2.12 kg/day FeS. This assumes that the rate at which Fe^{3+} can be reduced is not limiting.

The reduction of 1.36 kg/day Fe^{3+} equates to the reduction of approximately 500 kilograms per year of Fe^{3+}. Given an estimated total mass of 3,250 kilograms of iron in the saturated portion of the biowall, then the biowall matrix *potentially* has a 6.5 year supply of iron to sustain the production of FeS.

D.3.4.3 Accumulation of FeS

Based on the sulfate demand and available iron calculated in the preceding sections, the maximum amount of FeS that may accumulate can be estimated. Given a sulfate demand of 3.48 kg/day and an iron supply of 1.36 kg/day, then the amount of FeS that may be generated from the preceding calculations is approximately 2.12 kg/day.

The bulk density of the OU-1 mulch mixture is estimated to be 1.28 g/cm^3 based on a bulk density of the sand fraction of 1.66 g/cm^3 and a particle density of 0.9 g/cm^3 for the wood mulch. For the OU-1 mulch mixture with a total volume of 400 cubic yards (306 cubic meters), the mass of solid material in the saturated portion of the OU-1 biowall is approximately:

Mass of Solids = (1.28 g/cm^3) (306 m^3) (10^6 cm^3/m^3)

= 391,680,000 grams = 391,680 kilograms

FeS may accumulate at a rate of approximately:

Mass FeS per day = (2.12 kg/day) / (391,680 kg)

= 5.4 X 10-6 kg/day = 5.4 mg/day, or

= 1,970 mg/kg per year

The concentrations of AVS measured in the OU-1 biowall at 34 months after installation in April 2005 are listed in Table 4 of **Appendix F.2**. AVS is considered to be a measurement of the sulfide present in FeS in the biowall matrix. Concentrations of AVS ranged from 6,900 to 14,000 mg/kg, averaging approximately 10,400 mg/kg. Using AVS as a conservative measure of FeS, the field data suggest that FeS has accumulated at a faster rate of approximately 10 mg/kg per day. This is not inconsistent since the theoretical rate of accumulation (5.4 mg/day) is based on an estimated sulfate demand of 25 mg/L, where the supply of sulfate and iron are assumed to exceed the rate of consumption. The higher field observations may simply be due to a higher sulfate demand in the field at the OU-1 site.

Using a rate of generation of FeS of 2.12 kg/day, the potential to degrade TCE can also be evaluated. Based on the stoichiometric relationship where 0.3 milligram of FeS may potentially degrade 1.0 milligram of TCE (**Section D.3.3**), then the potential exists for up to 7 kilograms of TCE to be degraded per day. As shown in **Section D.3.2**, the estimated influx of TCE into the OU-1 biowall is 12.12 g/day. Based on stoichiometric relationships alone, the OU-1 biowall has the potential to degrade all the TCE migrating through the biowall by reaction with FeS. However, this does not account for the rate at which TCE may be degraded in the presence of FeS. Therefore, the mass of FeS required for effective degradation requires further evaluation.

D.3.5 Mass of FeS Required for Effective Abiotic Degradation

Shen and Wilson (2007) extracted rates of abiotic TCE degradation from column studies that ranged from 0.53 to 2.3 per day when exposed to 1.0 mole of FeS in contact with 1.0 liter of pore water ($d^{-1}M^{-1}$). This range of rates may be used with site-specific calculations to estimate the contribution to degradation of TCE from abiotic reactions with FeS.

If the bulk density of the mulch mixture is assumed to be 1.28 g/cm^3 (sand density of 1.66 g/cm^3 and mulch particle density of 0.9 g/cm^3), and the saturated portion of the OU-1 biowall contains 400 cubic yards of material (306 cubic meters), then the total amount of sulfide assumed to be present in the form of FeS (*i.e.*, AVS) may be approximated as follows:

Mass AVS = (10,400 mg/kg) (391,680 kg) = 4.073 X 10^9 milligrams = 4,073 kilograms

Where (from **Section D.3.4.3**):

Average concentration of AVS = 10,4000 mg/kg

Mass of solids = 391,680 kilograms

Given a total pore volume of 139,145 liters, then the amount of mineral sulfide per liter of water in the biowall is approximately 29.3 g/L.

Given a molar mass for sulfide of 32.06 g/L, then the concentration of sulfide (as AVS) measured for the OU-1 biowall is close to 1 mole per liter of pore water. It can be conservatively assumed that close to 1 mole of FeS per liter of pore water is present in the OU-1 biowall because the molar mass of FeS (87.91) is higher than sulfide alone. Therefore, it is reasonable to compare the rates calculated by Shen and Wilson (2007) to the extent of degradation of TCE observed at the OU-1 biowall.

Using a residence time of 15 days for the OU-1 biowall and the lower estimate of the rate constant for TCE of 0.53 $d^{-1}M^{-1}$ (**Section D.2.2**), a first-order law would predict that the concentration exiting the biowall would be 0.00035 of the influent concentration (a reduction of over 99 percent). Comparing concentrations immediately downgradient (5 feet) of the biowall relative to upgradient concentrations in Table 2 of **Appendix F.2** for April 2005, the reduction in concentration of TCE is over 99 percent; concentrations of TCE were below detection at 5 feet downgradient of the biowall. For locations approximately 30 feet downgradient of the biowall the average reduction is approximately 89 percent. Therefore, the reduction in TCE immediately downgradient of the biowall may be attributed to the presence of FeS in the biowall matrix and a normalized rate constant of 0.53 $d^{-1}M^{-1}$ of TCE in contact with 1.0 mole of FeS in 1.0 liter of groundwater. The relative reductions in TCE at 30 feet downgradient are less. This may be due to mixing with contaminated groundwater, desorption of TCE from aquifer solids, and/or diffusion of TCE from low permeability sediments.

D.3.6 Calculating Amendment Requirements to Enhance Production of FeS

Table D-2 includes inorganic amendments that may be used to increase the amount of sulfate or iron in biowall or bioreactor backfill material. Powdered gypsum was used at Dover AFB, Delaware. However, the product was difficult to mix with the other biowall materials as it formed a thick paste when hydrated by moisture in the mulch and sand. Gypsum fertilizer pellets were used at Ellsworth AFB, South Dakota, primarily due to ease of handling and ability to uniformly mix with the other biowall materials.

The easiest way to increase the amount of ferric iron is to screen local sources of construction sand for iron content. This was the approach used at Dover AFB, Delaware (Kennedy, 2004). Finding a suitable alternative amendment for ferric iron has proven more challenging than for sulfate. Iron ore may be procured, but at a substantial cost due to shipping and handling. The magnetite ore procured for Altus AFB and Ellsworth AFB was supplied by Reiss Viking. Other sources of hematite ore were available, but the cost increased substantially. Rusted scrap metal may be another potential source of ferric iron, if it can be processed to a size that will readily mix with the other biowall materials.

Table D-2 Potential Amendments to Stimulate the Formation of FeS

Common Name and Form	Applicable Compound	Composition	Percentage Sulfate or Iron	Bulk Cost (dollars)
Powdered Gypsum	Anhydrous calcium sulfate	$CaSO_4 \cdot 8H_2O$	34 percent sulfate	0.30 to 0.35 per pound
Gypsum Fertilizer Pellets	Anhydrous calcium sulfate	$CaSO_4 \cdot 8H_2O$	16 percent sulfate (typical)	0.15 to 0.20 per pound
Epsom Salts	Anhydrous magnesium sulfate	$MgSO_4 \cdot H_2O$	9.9 percent sulfate	1.00 per pound
Magnetite Ore	Magnetite	Fe_3O_4 (mineral magnetite)	64 percent total iron (640,000 mg/kg)	$800 per cubic yard, or $282 per ton
Iron sulfate (fertilizer/soil conditioner)	Ferrous iron sulfate, heptahydrate	$FeSO_4 \cdot 7H_2O$	18 percent sulfate and 30 percent ferrous iron (some commercial supplies are as low as 15% iron)	0.40 to 0.50 per pound in bulk

Attempts to stimulate biogeochemical transformation processes by adding a source of sulfate or iron have been conducted on an experimental basis at Dover AFB, Delaware; Ellsworth AFB, South Dakota; and Altus AFB, Oklahoma. For example, Parsons (2005b) evaluated the addition of iron and sulfate to a permeable mulch biowall at the BG05 Site at Ellsworth AFB, South Dakota. A series of spreadsheet calculations similar to those described in this appendix were used to quantify the amount of sulfate and iron in the natural system, and the amount of sulfate and iron that could be added with amendments. This was not intended as a rigorous design exercise, but rather to decide the appropriate materials and quantities to be added to a small portion (approximately 50 linear feet) of the BG05 biowall on an experimental basis. A number of simplifying assumptions were made in the calculations, and this represents just one approach to determining inorganic amendment requirements.

The material calculations assumed that the segment of the biowall to be amended with iron and sulfate is 50 feet long by 30 feet deep by 2 feet wide. The amended biowall segment was located in the most contaminated biowall transect, with an upgradient concentration of TCE of 175 μg/L. From a practical standpoint, the volume of gypsum pellets or iron sulfate fertilizer was limited to no more than 10 percent by volume of the biowall backfill material in the amended segment of the biowall. Similarly, the addition of iron ore (magnetite) was limited to 10 percent of the sand fraction of the biowall material.

The concentration of sulfate in groundwater at this site is approximately 400 mg/L, and the concentration of total iron in the sand that was procured was determined to be 5,100 mg/kg. To calculate the potential for degradation of TCE, it was assumed that 1) only 50 percent of the available sulfate is reduced to sulfide within the biowall, 2) only 10 percent of total iron is utilized per year (*i.e.*, a 10 year supply), and 3) only 20 percent of FeS that is formed reacts with TCE. The masses of TCE and sulfate in groundwater were based on a groundwater Darcy velocity (specific discharge) of 0.12 ft/day. The natural amounts of sulfate in groundwater and

iron in the biowall sand were then estimated to be sufficient to produce enough FeS to react with 60 times the estimated annual flux of TCE into the biowall. The system appeared to be iron limited based on the presence of a long-term, continuing source of sulfate in groundwater. Two alternatives were considered to enhance the potential for formation of FeS:

Alternative 1: Add 5.0 cubic yards of magnetite ore (14 tons, 64 percent iron content) and 9.6 cubic yards of gypsum fertilizer pellets (8,000 pounds, 16 percent sulfate content). This system would still be sulfate limited, but groundwater would provide a long-term, persistent source of sulfate.

Alternative 2: Add 8.4 cubic yards of iron sulfate fertilizer (7,000 pounds, 18 percent sulfate and 30 percent iron). This system would also appear to be sulfate limited; however, the iron in this product is soluble and may not persist.

It was understood that the iron in the iron sulfate fertilizer would be soluble, and it was thought that it would not persist for a long period of time. In addition, the effects on pH from using this product were not well understood. Therefore, a decision was made to add the magnetite ore and gypsum fertilizer pellets. This addition was estimated to roughly double the potential for accumulation of FeS in the biowall.

D.4 SUMMARY OF POTENTIAL FOR BIOGEOCHEMICAL TRANSFORMATION

The simplistic calculations and examples presented in this appendix should be used with caution. Documentation of biogeochemical reduction in the field has relied primarily upon indirect evidence including 1) reductions in concentrations of tetrachloroethene (PCE), TCE, and dichloroethene (DCE) without accumulation of dechlorination products DCE, vinyl chloride (VC), or ethene; and 2) measurement of the concentrations and valence state of iron and sulfide to estimate the concentration and mass of FeS in biowall or soil material. Data collected by Kennedy and Everett (2003) and Parsons (2007a) at the OU1 Biowall at Altus AFB, Oklahoma indicate that a sufficient mass of FeS was present to account for the reductions of TCE and DCE observed in groundwater, based on assumed stoichiometric relationships (see **Section D.3.4.3**).

However, laboratory studies suggest that not all forms of FeS may degrade chlorinated solvents equally, or at rates sufficient for environmental restoration (*e.g.*, Scherer, 2007). In addition, several sites where biogeochemical reduction should be significant (*e.g.*, the BG05 biowall at Ellsworth AFB, South Dakota and an emulsified vegetable oil application at Dugway Proving Ground, Utah [unpublished data]) suggest that biogeochemical reduction may not occur at measurable rates even under conditions suitable for the formation of FeS.

This suggests that the current protocols for measurement of bulk iron and sulfides in biowall material and soil may not always be sufficient to evaluate or predict the extent and rate of degradation of chlorinated solvents by reaction with FeS, potentially due to the following:

- Degradation and reaction rates of chlorinated solvents may be a function of mineral type, form (amorphous versus crystalline), and surface area.

- Formation and stability of appropriate mineral forms of FeS may be a function of prevailing geochemical conditions (*e.g.*, eh-pH phase or concentrations of other anions/cations in solution).

- Formation of suitable quantities of FeS may be a function of the mineralogy of the base sediment/materials present (mineral forms of native or emplaced iron, for example magnetite ore versus sand containing hematite).

- Recycling (reduction) of iron oxidized during biogeochemical reduction or by other geochemical reactions, particularly at the upgradient fringe of the reaction zone, may be required to sustain biogeochemical reduction. This infers that sulfate reduction also be sustained over time.

Tools to evaluate these potential factors may include more detailed petrographic analyses (*e.g.*, scanning electron microscope with electron microprobe), compound specific isotope analysis (CSIA), and geochemical modeling. AFCEE, along with the Environmental Security Technology Certification Program (ESTCP), the Naval Facilities Engineering Command (NAVFAC), and the United States Environmental Protection Agency (USEPA), continues to evaluate these factors as part of a biogeochemical transformation initiative (AFCEE *et al.*, 2008).

APPENDIX E

EXAMPLE PIPE CALCULATIONS

Appendix E: Example Pipe Calculations
Manifold Flow Distribution
Calculation, Assumptions, and Description
BG05 Biowall, Ellsworth AFB, South Dakota

Designed by: Parsons, 24 February 2004

The manifold included below the biowall, as specified in the Treatability Study work plan, is intended as an option to distribute additional carbon substrate in the event that the biowall alone is not sufficient to meet the desired performance goals. The following design package serves as the backup calculations for the selected design.

E.1 ASSUMPTIONS

1) The fluid injected through the pipeline will be either an emulsified oil and water mixture or lactic acid. Since the oil and lactic acid would both be carried by water and the typical ratios are from 2% to 4% oil or lactic acid, the physical properties of water at 60^{o}F are used for the calculations. It should be noted that as the fluid viscosity increases, the pressure loss along the length of the pipe increases and the difference in flow between the first and the last port may increase. This consideration is addressed in the Operational Considerations portion of this package.

2) The pipeline below the biowall will be a 3 inch diameter HDPE with a DR of 11. HDPE is a smooth pipe (ks=0).

3) The orifice discharge coefficient (Co) is for discharge to a static body of water. In this application, the orifice will discharge through the opening directly into the sand mulch mixture. Therefore, there will be head loss (and the resulting back-pressure on the orifice) as the injected fluid dissipates into the formation. It has been assumed that the pressure drop across the orifice will be the determining factor that specifies the flow per port. The system will be treated as if discharging into a static body of water.

4) The calculations for flow across an orifice are for a circular opening. The openings will actually be a slot in the pipe but pressure drop will be calculated for a circle of the same area. This will result in a conservative estimate since the hydraulic radius of the slot will be substantially lower then the hydraulic radius of a circle. The smaller the hydraulic radius will result in higher pressure loss across the orifice and better flow distribution along the pipeline. This improved distribution is not accounted for in the calculations because orifice coefficients could not be located for the slots specified. Note that although a specific reference is not available to confirm the assumption that a smaller hydraulic radius corresponds to a reduced flow rate, the use of this calculation is a standard in open channel flow.

5) An orifice is located at a single point along the pipeline and the length of pipe between them is not effected by the diameter of the orifice.

6) An injection pressure of 1 to 60 psi has been utilized for point injections of substrate. The pressure is required to overcome the static pressure of the water table and the head loss in the formation. A pressure anywhere in this range may be assumed for the static pressure inside the manifold at the last port.

7) The manifold will be installed at 30 feet below ground surface (bgs). The water table across the site is approximately 17 feet bgs. The greatest static pressure will be exerted on the manifold when the water table is at 17 feet bgs. This condition will be utilized to calculate the estimated flow across the pipeline.

8) The design will attempt to minimize the percent difference in discharge rate from the last port to the first port. In open water systems, a system is acceptable if the discharge (qn) from the last port is within 10% of the discharge from the first port. This system will be acceptable if the discharge from the last port is within 20% of the discharge from the first port. Both ends of the injection pipe will be accessible for injection so the fluid will be injected from both ends to compensate for the large flow variation.

E.2 CALCULATION DESCRIPTION

1) Utilize velocity boundary condition at Node 1 (N_1) of V_1=0.

2) Define the static pressure inside the manifold at N_1 (the last node). The pressure inside the manifold is referred to as P_1.

3) Calculate the headloss (h_1) across the last orifice (*Port 1*). See Equation 1.

4) Use the headloss at h_1 to calculate the flow through the first orifice (q_1). See Equation 2.

5) Calculate the velocity at Node 2 (N_2). See Equation 3.

6) Calculate the Reynolds Number (Re_2) at N_2 to determine the flow regime. See Equation 4.

7) Calculate friction head (h_{f2}). If Re<2,000 then use Equation 5 to calculate h_{f2}. If Re>3,000 then use Equation 6 to calculate h_{f2}. If 2,000<Re<3,000 then average the results of Equations 5 and 6.

8) Calculate the headloss (h_2) across the last orifice (*Port 2*). See Equation 8.

9) Use the headloss at h_2 to calculate the flow through the first orifice (q_2). See Equation 2.

10) Calculate the velocity at Node 3 (N_3). See Equation 3. This is the same as step (5). It is now possible to iterate steps (5) to (10) until the pressure is calculated that corresponds to zero (0) of the header length.

E.3 OPERATIONAL CONSIDERATIONS

1) An increase in fluid viscosity, due to the addition of carbon substrate (e.g., vegetable oil, lactate…), will result in increased pressure loss along the straight pipe run. This increased head loss could result in a flow difference between the first and last port that is greater than 20%. The ability to inject Substrate at both ends of the delivery system has been incorporated into the design to accommodate this variable.

2) Many assumptions were made during the design of this system. These assumptions were made to simulate the injection process. During the first injection performed using this system, the injection pressure should be monitored (standard) and the pressure at the last port should be monitored (not standard). The last port can be monitored by placing a pressure gauge on the end of the manifold not being used for injection. Additionally, the total flow rate injected into the system should be recorded. An actual system head curve for the distribution manifold can be developed with this data and the model can be calibrated to match the system performance by altering the fluid properties and the Orifice Coefficient.

3) The 3 inch HDPE pipe can be pressure jetted in the event of fowling.

4) Prior to using the pipe for injection, the injection substrate should be flushed into the entire pipe to insure that time is not spent displacing water present in the pipe.

5) After the pipe is used for substrate injection, if should be thoroughly flushed with water to decrease the risk bio-fowling.

Manifold Flow Distribution (continued)
Calculation, Assumptions, and Description

TABLE E.1 CALCULATION SUMMARY

P_1 (psi)	L (ft)	l (ft)	h_l Ratio	q_1 (gpm)	q_n (gpm)	q % Diff.	Q_{Total} (gpm)	P_S (psi)	Fill Time (hrs.)
Runs for lower pipe distribution manifold (3" DR 11, P_o = 13 ft H_2O, Trench Thickness = 2 ft, length from surface to first port = 40 ft)									
7[a/]	300	10	10.68	0.70	0.74	4%	22	7.1	9
10	300	10	12.37	1.27	1.32	3%	40	10.4	5
15	300	10	13.66	1.86	1.92	3%	58	15.8	3
20	300	10	14.28	2.30	2.37	3%	72	21..2	3

Notes:
P_1 - Pressure inside the manifold at the last port (user entered).
L - Total length of the diffuser (user entered).
l - Spacing between ports (user entered).
h_l Ratio- The ratio of the pressure head loss at the last port to the pressure head loss along the entire manifold.
Q_1 - Flow out of the last port.
Q_n - Flow out of the closest port.
Q % Diff. - The percent difference in flow from the last port (Q_1) to the first port (Q_n).
Q_{Total} - The total flow necessary for the system to operate at the pressure P_1.
P_S - The supply pressure necessary at the ground surface.
Fill Time - The time necessary to fill the pore volume of the trench with carbon source supplement.
[a/] - A printout of the complete calculation package is attached.

Manifold Flow Distribution (continued)
Calculation, Assumptions, and Description

Table E.2 Manifold Flow Distribution Input and Calculations

Note: User input values are shaded on this worksheet.

System Properties:

Specific Weight at 60°F (lb_f/ft^3) =	62.37	= γ			
Dynamic Viscosity at 60°F (lb_f s/ft^2) =	2.36E-05	= m			
Kinematic Viscosity at 60°F (ft^2/s) =	1.22E-05	= v			
Pipe Friction Factor =	0	= k_s			
Manifold Diameter for 3" DR 11 (ft) =	0.236	= D =	2.826	inches	
Depth to Water (ft bgs) =	17				
Depth to the Manifold (ft bgs) =	30				
Static Pressure Outside Manifold (ft H_2O) =	13	= P_o			
Effective Porosity of Trench Backfill =	20%				
Trench Width =	2.0	ft			
Length from the Surface to the First Port =	40	ft			

System Variables:

Static Pressure Inside Manifold at the last port (ft H_2O) =	16.15	= P_1 =	7	psi
Pressure Change Across Orifice (ft H_2O) =	3.15			
Manifold Area (ft^2) =	0.04			
Total Length of Diffuser (ft) =	300	= L		
Spacing Between Ports (ft) =	10	= l		

Discharge Area Calculations (calculation 1 or 2 is used for Flow Calculation):

Calculation 1, for slotted opening:

Slot Size =	0.020	inches		
Percent of pipe circumference slotted =	15%			
Discharge Area per Slot or Port Area (ft^2) =	0.00018			
Hydraulic Radius (ft) =	0.00250			

Calculation 2, for circular opening:

Port Diameter (ft) =	0.013888889	= d =	0.1666667	inches
Port Area (ft^2) =	0.00015			
Hydraulic Radius (ft) =	0.00344			

Flow Calculation for Last Orifice:

Port Area using Calc. 1 from above (ft^2) =	0.00018			
Orifice Coefficient =	0.6	= C^o		
Flow per Orifice (cfs) =	0.0016	= Q =	0.71	gpm

Manifold Flow Distribution (continued)
Calculation, Assumptions, and Description

Table E.3 Manifold Flow Distribution Calculation Results

Calculation Results, Analysis and System Summary:

Ratio of h_l Along Pipe to h_l Across Last Orifice =	10.68	(h_l = Pressure Head Loss)	
Flow out of the furthest port =	0.71	gpm	= Q_1
Flow out of the closest port =	0.74	gpm	= Q_n
Percent difference between closest and furthest port =	**4%**	is less then 20%.	
Total flow necessary for system =	22.24	gpm	= Q_{Total}
Supply pressure at closest port =	7	psi	= P_S

Head loss from surface to closest delivery point:

Fluid Velocity in Pipe (V) =	1.24	ft/s
Renolyds Number (Re) =	Turbulent	
friction resistance coefficient (f) =	0.0027	
Pipe Diameter (D) =	0.236	ft
Required Supply Pressure at Surface =	7.13	psi

The time to fill trench with a carbon supplement at the furthest port is 9 hours.

APPENDIX F
CASE STUDIES

APPENDIX F.1

BIOREMEDIATION OF CHLORINATED SOLVENTS USING A PERMEABLE MULCH BIOWALL SYSTEM AT THE ASH LANDFILL SITE, SENECA ARMY DEPOT ACTIVITY, NEW YORK

BIOREMEDIATION OF CHLORINATED SOLVENTS USING A PERMEABLE MULCH BIOWALL SYSTEM AT THE ASH LANDFILL SITE, SENECA ARMY DEPOT ACTIVITY, NEW YORK

Todd Heino, Jackie Travers and Beth Wasserman (Parsons, Boston, Massachusetts)
Bruce Henry (Parsons, Denver, Colorado)

1. INTRODUCTION

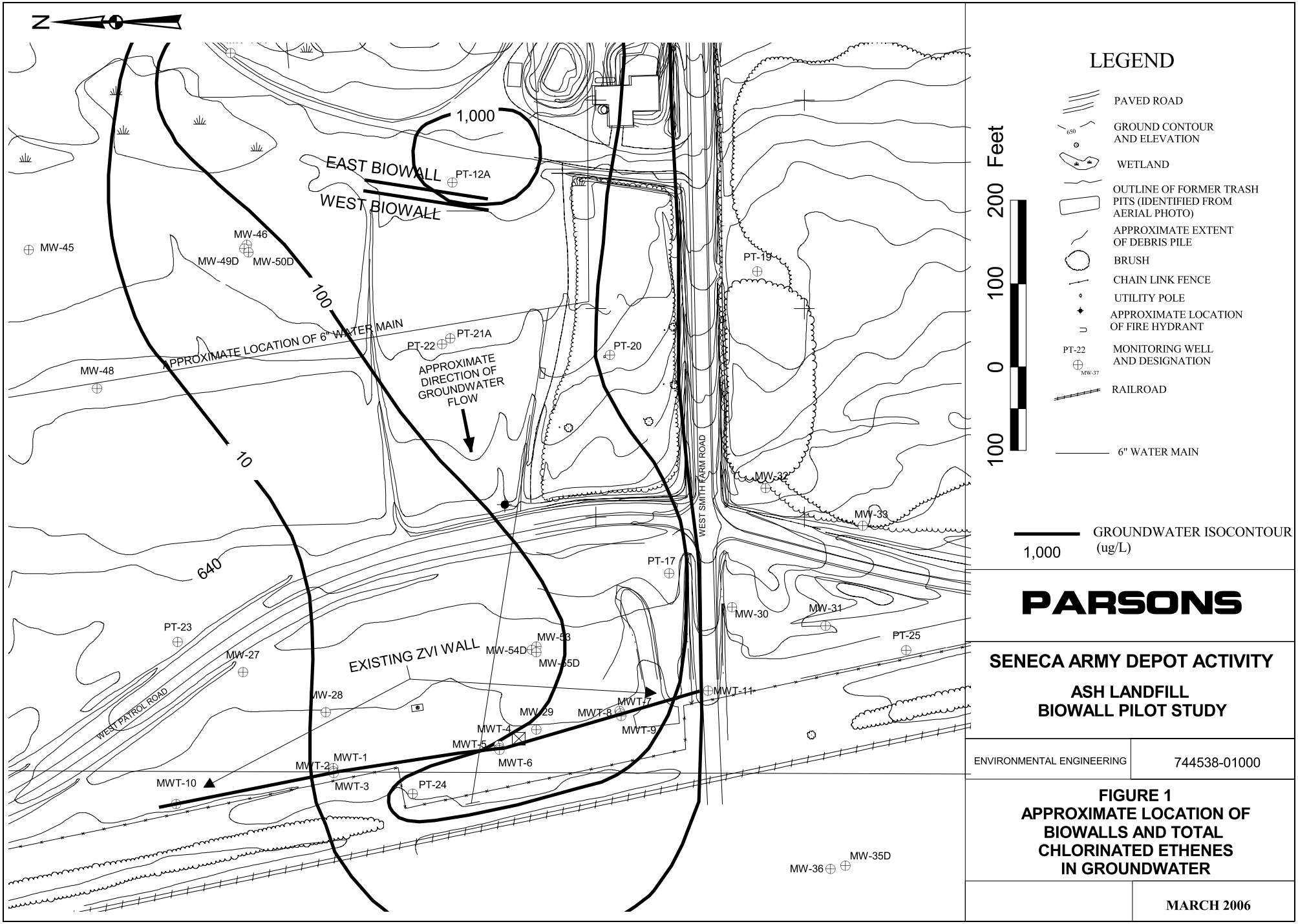
A permeable mulch biowall pilot test was used to enhance the *in-situ* bioremediation of chlorinated solvents at the Ash Landfill at Seneca Army Depot Activity, Romulus, New York. Two parallel biowalls were installed in August 2005 as a dual biowall system, and four rounds of sampling were conducted in September 2005, October 2005, December 2005 and January 2006. Based on pilot test results, the system has been expanded to full scale. This case study summarizes the installation and performance of the pilot test.

1.1 Objectives

The Ash Landfill biowall pilot test was used to stimulate the anaerobic biodegradation of chlorinated aliphatic hydrocarbons (CAHs, or chlorinated solvents) in groundwater. The biowalls were installed across the path of groundwater flow near the landfill source of a trichloroethene (TCE) groundwater plume (**Figure 1**). The primary objective of the pilot test was to demonstrate that a mulch biowall would be equally as effective, but cost less, than a pilot-scale zero-valent iron (ZVI) wall at the site in promoting the *in-situ* degradation of TCE, *cis*-1,2-dichloroethene (*cis*-DCE), and vinyl chloride (VC) in groundwater. Specifically, the pilot study was performed to demonstrate the following:

1. Achieve a similar or better reduction in concentrations of TCE within the biowall system relative to a ZVI wall previously installed downgradient of the mulch biowall pilot test (Parsons, 2000).
2. Demonstrate that the biowalls create a treatment zone within and downgradient of the trenches that is favorable to the long-term degradation of TCE and its regulated intermediate degradation products of DCE isomers and VC.
3. Demonstrate a reduction in total molar concentrations of CAHs in both the biowalls and at downgradient monitoring locations (*i.e.*, complete degradation and not just transformation from one chlorinated compound to another).
4. Demonstrate that CAHs will not exceed New York State Department of Environmental Conservation (NYSDEC) Groundwater Allowable (GA) Standards at a Farm House west of the site at any time during remediation of the site.
5. Evaluate biowall design criteria (*e.g.*, generation of organic carbon, degradation rates, residence time) and constructability issues (*e.g.*, trenching techniques, vegetable oil application, and subsurface pipe placement) required for effective long-term operation.

The long-term goal of using the biowall technology is to degrade CAHs to concentrations below the NYSDEC GA standards at a lower cost relative to expansion of the pilot-scale ZVI wall. The pilot study objectives have been met and the Army has proceeded with design and installation of a full-scale biowall application as the final remedy for the Ash Landfill Site.



PARSONS

SENECA ARMY DEPOT ACTIVITY

ASH LANDFILL
BIOWALL PILOT STUDY

ENVIRONMENTAL ENGINEERING	744538-01000

FIGURE 1
APPROXIMATE LOCATION OF BIOWALLS AND TOTAL CHLORINATED ETHENES IN GROUNDWATER

MARCH 2006

O:\Seneca\Ash_lf\Ash RD WP\ash_rd_wp.apr\Fig 2-Ash base Plume & Biowall

1.2 Technology Description

The permeable mulch biowall is intended to stimulate the complete anaerobic reductive dechlorination of TCE in groundwater at the Ash Landfill Site. Solid-phase organic substrates used to stimulate anaerobic biodegradation of CAHs include plant mulch and compost. Mulch is primarily composed of lignin, cellulose, and hemicellulose. Typically the mulch is partially composted, or compost is added, to provide a source of nutrients and more readily degraded organic carbon for microbial growth. These substrates are mixed with coarse sand or gravel and emplaced in a trench or excavation in a permeable biobarrier configuration. Vegetable oil may also be added to the mulch mixture to increase the amount of readily bioavailable organic matter.

This treatment method relies on the flow of groundwater under a natural hydraulic gradient through the biowall to promote contact with slowly-degraded organic matter. As the groundwater flows through the organic matter within the biowall, a treatment zone is established within and downgradient of the biowall as anaerobic microbial processes are stimulated. A mulch biowall has the potential to stimulate reductive dechlorination of CAHs for many years. If needed, mulch biowalls can be periodically recharged with fluid substrates (*e.g.*, emulsified vegetable oil) to extend the life of the biowall remedy.

1.3 Scope of Work

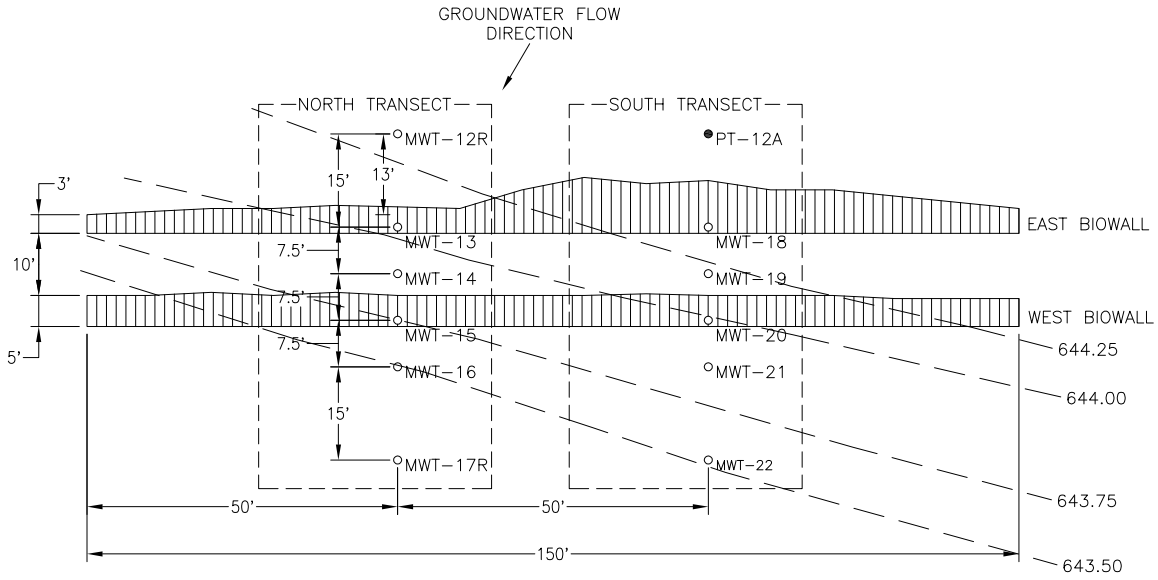
Site-specific activities conducted at the Ash Landfill in support of the enhanced bioremediation application included the following:

- Installation from 18 to 22 July 2005 of two parallel 150-foot long, by 11-foot deep, by 3.0-foot wide mulch biowalls composed of shredded tree mulch and sand (**Figure 2**). The mulch/sand mixture in the East Biowall was coated with soybean oil prior to placement in the trench;
- Installation of 11 groundwater monitoring wells in August 2005;
- Post-installation sampling of groundwater at the newly installed monitoring wells and existing monitoring well PT-12A in September 2005, October 2005, December 2005 and January 2006; and
- Aquifer testing of the newly installed monitoring wells to estimate hydraulic conductivity.

In addition to the wells shown in **Figure 2**, Well MW-39 (upgradient of the biowall system) and well PT-22 (located 150 feet downgradient of the biowalls) were sampled on 01 December 2005 to provide supplemental data. MW-39 and PT-22 were both sampled during the December 2005 sampling event (Round 3), and PT-22 was sampled again during the January 2006 sampling event (Round 4).

Groundwater samples collected after installation of the biowall system were analyzed for volatile organic compounds (VOCs), dissolved oxygen (DO), nitrate, nitrite, ferrous iron, manganese, sulfate, sulfide, carbon dioxide, methane, ethane, ethene, oxidation-reduction potential (ORP), alkalinity, pH, temperature, specific conductance, total organic carbon (TOC), volatile fatty acids (VFAs), and chloride.

Plan View of As-Built Biowall



LEGEND

- ●PT-12A 2" GROUNDWATER MONITORING WELL INSTALLED PRE-BIOWALL INSTALLATION
- ○MWT-17 2" GROUNDWATER MONITORING WELL INSTALLED POST-BIOWALL INSTALLATION
- BIOWALL

20' 10' 0 20'

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ASH LANDFILL
BIOWALL PILOT STUDY

ENVIRONMENTAL ENGINEERING	744538-01100

FIGURE 2
GROUNDWATER ELEVATIONS IN BIOWALL MONITORING NETWORK
SEPTEMBER 7, 2005

1" = 20'	MARCH 2006

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2. SITE DESCRIPTION

The Ash Landfill site includes a groundwater plume that emanates from the northwestern side of the landfill area and extends approximately 1,100 feet from the source area to the western Depot property line. The plume consists of primarily of TCE and *cis*-DCE. A remedial investigation/feasibility study (RI/FS) was completed in 1996, and a Non-Time Critical Removal Action (NTCRA) was conducted by the Army between August 1994 and June 1995 to remove contaminated material the landfill source area. The NTCRA involved the excavation of 63,000 cubic yards of soil and treatment using low temperature thermal desorption. The surface area involved approximately 1.5 acres. The NTCRA provided a positive benefit for the long-term site restoration by eliminating the continued leaching of CAHs into groundwater and preventing further exposure to humans and wildlife. In the several years since the removal action, contaminant concentrations in groundwater in the source area have decreased by over two orders of magnitude.

A ZVI wall treatability study was performed between 1998 and 2001, and showed that a ZVI wall would degrade chlorinated ethenes (*i.e.*, TCE, *cis*-DCE, and VC). A 650-foot long by 15-foot deep by 14-inch wide trench was excavated near the Depot property line and backfilled with a 50/50 mix of granular ZVI and sand. Monitoring was conducted from 1999 to 2004 to assess the performance of the ZVI wall. A Record of Decision (ROD) for this site was subsequently issued in February 2005 (Parsons, 2005) and included the use of permeable reactive walls as migration control for groundwater contamination on site.

The site is underlain by a broad north-to-south trending series of rock terraces covered by a mantle of glacial till. As part of the Appalachian Plateau, the region is underlain by a tectonically undisturbed sequence of Paleozoic rocks consisting of shales, sandstones, conglomerates, limestones and dolostones. At the Ash Landfill site, these rocks (the Ludlowville Formation) are characterized by gray calcareous shale and mudstone, and thin limestones with numerous zones of abundant invertebrate fossils. Locally, the shale is soft, gray, and fissile. Pleistocene age till deposits overlie the shales, which have a thin (2 to 3 feet) weathered zone at the top. The till matrix varies locally but generally consists of poorly sorted silt, clay, sand, and gravel. At the Ash Landfill Site, the thickness of the till generally ranges from 4 to 15 feet. At the location of the biowalls, the thickness of the till and weathered shale is approximately 10 to 15 feet.

Groundwater is present in both the shallow till/weathered shale and in the deeper competent shale. In both water-bearing units, the predominant direction of groundwater flow is to the west, toward Seneca Lake. Based on historical data, groundwater in the till/weathered shale exhibits rhythmic, seasonal fluctuations in water table elevation and saturated thickness. The saturated interval is at its thinnest (generally between 1.0 and 3.0 feet thick) in the month of September, and is the thickest (generally between 6 and 8.5 feet thick) between the months of December and March.

The average linear velocity of groundwater in the till/weathered shale was calculated during the RI (Parsons, 2004) using the following parameters: 1) an average hydraulic conductivity of 4.5×10^{-4} centimeters per second (cm/sec) (1.28 feet per day [ft/day]), 2) an estimated effective porosity of 15 to 20 percent, and 3) a groundwater gradient of 1.95×10^{-2} foot per foot (ft/ft). The average linear velocity was calculated to 0.166 ft/day or 60.7 feet per year (ft/yr) at 15 percent effective porosity, and 0.125 ft/day or 45.5 ft/yr at 20 percent effective porosity. The maximum groundwater velocity at the pilot test location may an order of magnitude or more higher in more permeable zones associated with heterogeneity in the till/weathered shale.

The average linear velocity of the groundwater in the competent shale was calculated using the following parameters: 1) an average hydraulic conductivity of 3.73 x 10^{-5} cm/sec (0.106 ft/day), 2) an estimated effective porosity of 6.75 percent (0.0675), and 3) a groundwater gradient of 2.5 x 10^{-2} ft/ft. An average linear velocity of 3.9 x 10^{-2} ft/day or 14.3 ft/yr was calculated for the competent shale.

TCE and *cis*-DCE are the most prevalent CAHs in both extent and concentration in groundwater at the Ash Landfill. The areal extent of total chlorinated ethenes based on groundwater samples collected in January 2000 is illustrated in **Figure 1**. Subsequent monitoring has shown little change since then. The plume originates from the Ash Landfill and extends west approximately 1,100 feet to the Depot's western boundary. Concentrations of total chlorinated ethenes in January ranged up to 2,088 micrograms per liter (µg/L). The plume is controlled to a limited extent by the 650-foot long permeable reactive ZVI wall installed upgradient of the Depot property line.

3. BIOWALL SYSTEM CONSTRUCTION

Two biowalls were constructed perpendicular to the path of groundwater flow in the vicinity of monitoring well PT-12A, as shown in plan view on **Figure 2**. The area selected for installation has historically shown the highest concentrations of chlorinated ethenes. The East Biowall is 150-foot-long and averages 11.3 feet deep and 3.0 feet wide. Due to some sloughing of the trench sidewalls, some areas of the biowall are as much as 6.0 feet wide. The West Biowall is 150-feetlong and averages 10.7 feet deep and 3.0 feet wide. The walls were installed 15 feet apart. A total of 200 cubic yards of shredded mulch and 150 cubic yards of sand was mixed and backfilled into the trenches. The mulch consisted of shredded plant material (a mix of whole deciduous and evergreen trees).

The mulch/sand mix for the West Biowall was coated with 880 gallons of soybean oil prior to placement to evaluate if it would enhance the effectiveness of the mulch mixture. Additionally, a 3-inch high density polyethylene (HDPE) pipe was installed in the West Biowall for future injection of fluid substrates or amendments, if required to maintain biowall performance.

An excavator was employed to excavate the trench for the biowall (**Figure 3**). The excavator utilized rock teeth to properly key the bottom of the trench through the fractured bedrock into the competent bedrock. The backfill material was placed in the trench using a front-end loader. Soil generated during the excavation was temporarily stockpiled next to the biowall. The final disposition of the soil was dependent on confirmation sampling for concentrations of TCE, and soils were ultimately spread on site.

Figure 3. Installation of the Ash Landfill Biowalls

Following construction of the biowall, 11 groundwater monitoring wells were installed to form two monitoring well transects perpendicular to the biowalls along the direction of groundwater flow. Existing well PT-12A was used as the upgradient well for the southernmost transect. Wells were installed 15 feet upgradient of the East Biowall, within the footprint of each biowall, between the biowalls and at distances of 7.5 and 15 feet downgradient (to the west) of the biowalls. These points are used to monitor groundwater geochemical indicators and contaminant concentrations within, between, and downgradient of the biowall system.

4. MONITORING RESULTS

Monitoring results from the four rounds of sampling are presented in the following subsections on hydrogeology, geochemistry, substrate and electron donor distribution, and degradation of chlorinated ethenes. The results are intended to show that the biowalls have altered groundwater geochemistry to promote sequential reductive dechlorination of TCE and *cis*-DCE to VC and ethene. Two transects of monitoring wells are located along the path of groundwater flow, perpendicular to the two biowall trenches (**Figure 2**). The North Transect consists of wells MWT-12R through MWT-17R. The South Transect consists of wells PT-12A and MWT-18 through MWT-22. Monitoring well PT-22 was also added to the last two rounds of sampling to assess performance biowall further downgradient of the biowalls (approximately 150 feet downgradient of the biowalls).

4.1 Hydrogeology

Depth to groundwater within the East Biowall ranged from approximately 2.2 to 6.7 feet below ground surface (bgs), while depth to groundwater within the West Biowall ranged from approximately 2.5 to 7.4 feet bgs. The depth of the eastern trench averages 11.3 feet bgs and the depth of the western trench is an average of 10.7 feet bgs. Therefore, the saturated thickness within the two biowall trenches ranges from 3.3 to 9.1 feet at any given time, depending on seasonal changes in groundwater levels due to recharge from precipitation. **Figure 2** includes contours of the shallow groundwater potentiometric surface for September 1, 2005 (Round 1)**.**

The biowalls were installed to the top of the competent shale (bedrock) surface. The biowall trenches do not intercept the entire width of the CAH groundwater plume as the trenches were installed as a pilot test only. Therefore, mixing of treated groundwater from the biowall and contaminated groundwater downgradient of the biowall trench will occur to some degree. Monitoring results for well locations more than 10 to 20 feet downgradient of the biowall should be evaluated with the understanding that not all of the water at those monitoring locations may have passed through the biowall. Results for wells MWT-13, MWT-15, MWT-18 and MWT-20, located within the biowall trenches, are the most representative of the degree to which the biowalls are effective in remediating CAHs in groundwater passing through the biowalls.

The groundwater surface slopes northwest toward Seneca Lake, with horizontal hydraulic gradients ranging from 0.03 ft/ft to 0.05 ft/ft at the North Transect and ranging from 0.02 ft/ft to 0.03 ft/ft at the South Transect. Hydraulic conductivity in the till/weathered shale formation ranges from 5.1E-5 to 1.6E-4 cm/sec in the North Transect and ranges from 2.0E-5 to 2.5E-4 cm/sec in the South Transect. The hydraulic conductivity measured in the biowall were an order of magnitude greater than those measured in the till/weathered shale formation, ranging from 1.9E-3 to 2.8E-3 cm/sec in the North Transect and ranging from 1.0E-3 to 7.3E-3 cm/sec in the South Transect.

Using the range of calculated hydraulic conductivity derived from the slug test data, the horizontal hydraulic gradients, and an estimated effective porosity of 15 percent, the advective velocity of groundwater flow in the till/weathered shale formation exiting the biowalls ranges from approximately 0.028 to 0.071 ft/day (10 to 26 ft/yr) in the North Transect, and ranges from approximately 0.010 to 0.14 ft/day (4.0 to 53 ft/yr) in the South Transect. The velocities of groundwater exiting the East Biowall along each transect were calculated by considering the hydraulic gradient between the monitoring wells at the western edge of the biowall (MWT-13 and MWT-18) and the monitoring wells immediately downgradient of the East Biowall (MWT-14 and MWT-19).

Observations of geochemical parameters monitored over the duration of the test indicate that the groundwater advective velocity may be greater than aquifer (slug) test results indicate. Based on the time it took for breakthrough of geochemical parameters to be observed at the downgradient wells, it appears that flow through the North Transect may be on the order of 100 ft/yr, and flow through the South Transect may be between 200 and 400 ft/year. Based on these groundwater velocities, the residence time through the biowall system (approximately 18 feet) is approximately 66 days for the North Transect and between 16 and 33 days for the South Transect. Calculation of residence time does not account for the effects of a higher effective porosity with the biowall itself, nor do they account for sorption of contaminants onto the mulch matrix. Therefore, groundwater residence time calculations are considered a conservative estimate of the residence time of CAHs within the biowall trench.

4.2 Groundwater Geochemistry

Biodegradation causes measurable changes in groundwater geochemistry that can be used to evaluate the effectiveness of substrate addition. For anaerobic reductive dechlorination to be an efficient process, the groundwater typically must be sulfate-reducing or methanogenic. Thus, groundwater in which anaerobic reductive dechlorination is occurring should have the following geochemical signature:

- Depleted concentrations of DO, nitrate, and sulfate;
- Elevated concentrations of ferrous iron, manganese, methane, carbon dioxide, chloride, and alkalinity; and
- Reduced ORP.

Selected geochemical parameters are shown on **Table 1**. Comparison of geochemical parameters for biowall well locations MWT-13 and MWT-18 (East Biowall) and MWT-15 and MWT-20 (West Biowall) to well locations outside the biowall are summarized below.

Dissolved Oxygen. DO is the most favored electron acceptor used by microbes for the biodegradation of organic carbon, and its presence can inhibit the biodegradation of CAHs. DO levels were already naturally depleted, being less than 2.0 milligrams per liter (mg/L) in the study area. In the last round of sampling (January, 2006), concentrations of DO were less than 0.30 mg/L at all sample locations up to 150 feet downgradient of the biowalls.

TABLE 1
GROUNDWATER GEOCHEMICAL DATA

Sample Location	Sample Date	Temp (^oC)[a/]	pH (su)[b/]	Total Organic Carbon (mg/L)[c/]	ORP (mV)[d/]	Dissolved Oxygen (mg/L)	Manganese (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Sulfide (mg/L)	Alkalinity (mg/L)	Methane (ug/L)[e/]	Ethane (ug/L)	Ethene (ug/L)
MW-39	02-Dec-05	10.7	7.19	<1.0	76	0.31	<0.1	0.11	27.2	0.05	212	0.79	0.006 J[f/]	<0.025
(Background)	16-Dec-05	--[g/]	--	--	--	0.09	--	--	--	--	--	--	--	--
PT-22	02-Dec-05	9.9	6.98	7.8	57	1.00	1.4	4	110	0.02	413	110	0.017 J	10
(150' Downgradient	16-Dec-05	10.2	7.00	13	-44	0.08	0.8	0.1	88.8	0.01	649	990	0.14	45
of the biowalls)	24-Jan-06	7.0	7.28	6.9	-91	0.10	1.5	0.17	78.3	0.01	472	970	0.3	30
South Transect														
PT-12A	07-Sep-05	18.5	7.14	4.7	50	0.96	0.3	0.04	325	0	313	1.1	0.1	0.066
(15' Upgradient)	24-Oct-05	13.1	6.88	4.0	32	0.00	0.5	0.17	390	0	420	11	0.17	0.18
	12-Dec-05	9.7	7.03	2.6	84	0.41	0.3	0.3	515	0.01	306	15	0.15	0.2
	24-Jan-06	7.0	7.25	4.2	93	0.39	1.1	0.16	585	0	320	26	0.18	0.25
MWT-18	07-Sep-05	22.9	6.57	1990	-178	1.25	>22[h/]	4.7	71.7	15.4	2,630	4,600	0.52	0.55
(In East Biowall)	24-Oct-05	16.1	6.44	777	-177	<0.01	>22	2.51	<2.0	0.19	1,700	14,000	0.054	0.084
	12-Dec-05	10.8	6.62	918	-137	0.10	>22	2.49	<10	0.15	1,420	11,000	0.039	0.72
	24-Jan-06	8.2	6.62	4.2	-151	0.06	>22	3.11	<4.0	0.26	1,430	19,000	0.29	2.7
MWT-19	07-Sep-05	22.0	7.74	208	-145	2.19	12.4	5.1	492	0.05	846	98	0.18	0.46
(Between biowalls)	24-Oct-05	14.3	6.79	42	-226	0.00	5.6	>3.30	150	0.04	940	1,100	0.29	0.67
	12-Dec-05	8.0	7.00	48	-114	0.74	3	2.04	148	0.03	999	2,100	0.37	7.5
	24-Jan-06	7.6	6.91	74	-256	0.06	7.4	>3.30	80.3	0.07	1,145	3,850	0.55	115
MWT-20	07-Sep-05	22.2	7.70	951	-197	0.12	13.2	2.73	<2.0	0.54	2,480	7,700	0.04	0.22
(In West Biowall)	24-Oct-05	17.0	7.22	268	-212	1.07	11.9	>3.30	<2.0	0.3	2,350	13,000	0.01J	0.54
	12-Dec-05	10.2	6.76	173	-149	0.07	>22	2.47	<4.0	0.14	917	12,000	0.042	11
	24-Jan-06	7.0	6.76	25	-171	0.07	>22	>3.30	<4.0	0.11	995	18,000	0.35	16
MWT-21	07-Sep-05	19.8	7.85	165	-245	0.44	15.8	4.1	443	0.632	118	1,000	0.45	0.78
(7.5' Downgradient)	24-Oct-05	15.4	7.19	113	-275	1.22	9.4	>3.30	156	0.11	1,090	3,300	0.26	1.7
	12-Dec-05	9.3	6.80	70	-235	0.04	0.6	2.06	199	-	1,500	6,100	0.38	83
	24-Jan-06	7.3	8.02	54	-273	0.10	10.9	2.41	114	0.28	940	11,000	0.85	100
MWT-22	07-Sep-05	17.8	8.10	361	-180	0.45	22	4.73	278	0.269	1,030	1,300	1.7	3.4
(22.5' Downgradient)	24-Oct-05	13.6	7.35	33	-228	1.28	6.1	2.68	296	0.04	1,115	1,900	1.2	3.5
	12-Dec-05	9.0	6.82	35	-206	0.04	0.7	2.27	282	0.06	861	1,900	1.2	95
	24-Jan-06	8.3	6.72	36	-104	0.15	6.1	2.3	370	0.05	731	2,300	1.2	93

(continued)

TABLE 1 (Continued)
GROUNDWATER GEOCHEMICAL DATA

Sample Location	Sample Date	Temp (°C)[a/]	pH (su)[b/]	Total Organic Carbon (mg/L)[c/]	ORP (mV)[d/]	Dissolved Oxygen (mg/L)	Manganese (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Sulfide (mg/L)	Alkalinity (mg/L)	Methane (ug/L)[e/]	Ethane (ug/L)	Ethene (ug/L)
North Transect														
MWT-12R	07-Sep-05	22.1	7.32	7.3	10	1.67	1.0	0.41	732	0.01	304	23	0.35	1.52
(15' Upgradient)	24-Oct-05	13.7	6.86	4.9	27	<0.01	0.8	0.05	767	0.01	800	97	0.63	2.25
	12-Dec-05	8.4	6.92	3.7	36	0.84	1.0	0.22	903	0.1	301	140	1.3	3.6
	24-Jan-06	7.4	6.95	3.8	54	0.56	1.0	0	741	0.03	296	150	0.85	2.7
MWT-13	07-Sep-05	20.5	6.01	296	-220	0.00	>22	0.01	<20	0.61	183	3,100	0.5	0.93
(In East Biowall)	24-Oct-05	15.4	6.47	1,310	-158	0.00	>22	2.81	<2.0	0.24	2,530	10,000	0.11	0.15
	12-Dec-05	10.6	6.55	588	-169	0.06	>22	3.15	<4.0	0.2	10 U	12,000	<0.025	0.8
	24-Jan-06	7.4	6.54	298	-150	0.11	>22	>3.30	<4.0	0.19	731	14,000	0.078	6.8
MWT-14	07-Sep-05	21.1	6.72	610	-177	<0.01	>22	0.04	631	0.1	1,240	31	0.15	0.26
(Between biowalls)	24-Oct-05	14.8	7.19	432	-252	1.08	>22	>3.30	69.9	0.11	1,450	6,100	0.1	0.34
	12-Dec-05	11.5	6.30	275	-165	0.17	>22	>3.30	53.8	0.13	1,170	14,000	0.22	89
	24-Jan-06	6.7	6.59	209	-113	0.15	>22	2.7	51.9	0.18	879	14,000	2.4	190
MWT-15	07-Sep-05	20.6	6.90	1,060	-199	0.00	>22	5.1	<4.0	0.31	2,020	8,100	0.031	0.28
(In West Biowall)	24-Oct-05	16.5	7.27	267	-206	1.05	17.6	2.81	<2.0	0.16	1,900	10,000	<0.008	1.9
	12-Dec-05	11.1	6.28	87	-159	0.06	>22	2.61	<10.0	0.14	774	17,000	0.99	16
	24-Jan-06	6.5	6.76	47	-150	0.16	>22	2.44	33.2	0.09	515	28,000	4.3	15
MWT-16	07-Sep-05	20.4	7.10	64	-119	1.70	1	0.83	345	0.3	551	23	0.081	0.14
(7.5' Downgradient)	24-Oct-05	14.4	7.13	204	-175	1.35	7.3	2.24	2	0.13	1,300	4,800	0.19	2.2
	12-Dec-05	10.7	6.45	89	-160	<0.01	>22	>3.30	16.9	0.14	1,050	6,200	0.68	72
	24-Jan-06	7.9	6.65	52	-128	0.18	>22	2.58	27.8	0.02	929	11,000	5.3	120
MWT-17R	07-Sep-05	20.7	7.28	9.3	60	1.25	0.1	0	408	0.7	351	1.1	0.085	0.21
(22.5' Downgradient)	24-Oct-05	13.8	6.75	111	-27	<0.01	5.2	0.2	80.5	0.1	1,005	1,000	0.049	0.58
	12-Dec-05	8.7	6.39	64	-126	<0.01	3.3	0.8	43.8	0.08	1,180	4,700	0.38	42
	24-Jan-06	6.7	7.56	30	-156	0.29	15.2	>3.30	58.5	0.07	781	7,300	1.4	51

[a/] °C = degrees Centigrade.

[b/] su = standard pH units.

[c/] mg/L = milligrams per liter.

[d/] mV = millivolts.

[e/] µg/L = micrograms per liter.

[f/] J-flag indicates the concentration is below the quantification limit but above the method detection limit, and the concentration is estimated.

[g/] "--" indicates parameter could not be measured.

[h/] >22 indicates the measurement is over the range of detection indicated for the test method.

Oxidation-Reduction Potential. Low ORP, less than -100 millivolts (mV), is typically required for anaerobic reductive dechlorination to occur. Through the first two rounds of sampling, ORP upgradient of the biowall has ranged from +10 mV to +100 mV, indicating background conditions are only mildly anoxic. Within the East and West Biowalls, ORP has been lowered to a range of –137 mV to –220 mV. These levels of ORP indicate conditions are sufficiently reducing within the biowalls to support sulfate reduction, methanogenesis, and anaerobic reductive dechlorination. By January 2006, all monitoring locations downgradient of the biowalls (to a distance of 22.5 feet) exhibited ORP of less than -100 mV, indicating that highly reducing conditions are present over a large area downgradient of both biowalls as well.

Ferrous Iron. Ferric iron (III) may be used as an electron acceptor during anaerobic biodegradation of organic carbon. During this process, iron (III) is reduced to soluble ferrous iron (II), which can be measured in groundwater samples. Concentrations of iron (II) upgradient of the biowall are less than 0.5 mg/L. Within the biowall, concentrations of iron (II) are elevated, with a maximum concentration of 5.1 mg/L measured at location MWT-15 in October 2005. Several readings of iron (II) were reported as >3.3 mg/L due to the upper detection limit of the field reagent used. The elevated concentrations are maintained in all downgradient locations. Elevated concentrations were not evident in PT-22, 150 feet downgradient of the biowalls. Iron (II) levels remain close to background at this location.

Sulfate. Sulfate is used as an electron acceptor during sulfate reduction, competing with anaerobic reductive dechlorination for available substrate (electron donor). Sulfate levels lower than 20 mg/L are desired to prevent inhibition of reductive dechlorination of chlorinated ethenes. However, elevated levels of sulfate and iron may be beneficial for stimulating biogeochemical reduction by the formation of reactive iron sulfides (*e.g.*, Butler and Hayes, 1999; Lee and Batchelor, 2002). Sulfate levels upgradient of the biowalls range from 325 to 903 mg/L. By the second round of sampling, the levels of sulfate were depleted to non-detect levels within the biowalls, except for the January 2006 round in MWT-15 (33 mg/L).

Methane. The presence of methane in groundwater is indicative of strongly reducing methanogenic conditions, optimal for anaerobic reductive dechlorination to occur. Methane concentrations in the two upgradient wells range from 0.001 mg/L to 0.15 mg/L. Concentrations of methane measured in the biowalls were elevated at 3.1 mg/L to 8.1 mg/L in September 2005, and increased to 14 mg/L to 28 mg/L in January 2006. Methane levels in the downgradient wells (1.0 mg/L to 11 mg/L) are significantly higher than upgradient wells for the October 2005 through January 2006 sampling rounds.

4.3 Substrate Distribution and Electron Donors

The distribution of soluble organic substrate in groundwater may be reflected in elevated levels of TOC and metabolic acids measured in groundwater. The presence of organic substrate is necessary to fuel anaerobic degradation processes.

Total Organic Carbon. During the first three rounds of sampling, concentrations of TOC in the wells within the biowalls (87 mg/L to 1,990 mg/L) were two orders of magnitude higher than upgradient of the biowalls (2.6 mg/L to 7.3 mg/L). Levels within the biowalls in the North Transect decreased during the third and fourth sampling rounds. For example, levels of TOC decreased from 1,990 mg/L in MWT-18 to 4.2 mg/L and from 951 mg/L in MWT-20 to 25 mg/L. However, levels apparently remain sufficient to maintain sulfate reducing and methanogenic conditions. TOC levels remain elevated in the wells downgradient of the biowalls, ranging from 30 mg/L to 36 mg/L in the January 2006

sampling round at the wells located 22.5 feet downgradient of the biowalls (MWT-22 and MWT-17R).

Metabolic Acids. Metabolic acids, or VFAs, are produced during the biodegradation of organic substrates (*e.g.*, produced by sulfate reducers). An increase in metabolic acids is an indication that microbial activity has been stimulated. These metabolic acids may be further fermented to produce molecular hydrogen, the primary electron donor utilized during reductive dechlorination of chlorinated ethenes. Metabolic acids (data not shown) measured were comprised primarily of acetic, pentanoic, propionic, and butyric acids. Total metabolic acids were less than 2.0 mg/L in the upgradient wells. Total metabolic acid concentrations increased to between 60 mg/L to 7,926 mg/L within the biowalls. In the South Transect downgradient wells, metabolic acid concentrations ranged from 316 to 820 mg/L in September 2005, and decreased to between 4 and 34 mg/L in January 2006. In the North Transect, concentrations ranged from 91 to 161 mg/L in October 2005, and decreased to between 8 to 23 mg/L in January 2006. The decrease in metabolic acid production over time correlates to a decrease in TOC concentrations over time.

In summary, levels of TOC and metabolic acids were highly elevated immediately after installation of the biowall. This is likely due to the dissolution of the soluble portion of organic matter that was present in the mulch added to the biowall trenches. Levels of TOC and metabolic acids appear to have stabilized to more sustainable levels. In addition, as the microbial community grows it is capable of utilizing the available organic carbon more rapidly, and less organic carbon migrates out of the immediate biowall treatment zone. It is not yet known what levels of substrate the biowall will be able to sustain over the expected design life of 5 years or more, or what threshold concentrations are required to sustain effective biodegradation. As of January 2006, the effectiveness of the biowall system continues to increase with time as the microbial community adapts to anaerobic conditions.

4.4 Degradation of Chlorinated Ethenes

Table 2 summarizes VOCs detected in groundwater during monitoring of the Ash Landfill biowall pilot study. The first round of groundwater sampling was performed approximately 4 weeks after installation of the biowall. While true "baseline" conditions for the wells located in the trenches and downgradient were not obtained, data from upgradient wells PT-12A and MWT-12R can be used to infer "baseline" conditions.

4.4.1 Trends in Chlorinated Ethene Concentrations

The primary contaminants detected at the site include TCE, *cis*-DCE, and VC. During the four sampling rounds, upgradient concentrations of TCE ranged from 400 μg/L to 860 μg/L, and upgradient concentrations of *cis*-DCE ranged from 310 μg/L to 980 μg/L. Concentrations of VC detected upgradient of the biowall system ranged from <1.2 μg/L to 24 μg/L in the South Transect (PT-12A), and from 64 μg/L to 86 μg/L in the North Transect (MWT-12R). Lower concentrations (less than 25 μg/L) of *trans*-1,2-DCE and 1,1-DCE have also been detected in upgradient monitoring locations PT-12A and MWT-12R.

As of the second monitoring event in October 2005, a trend of decreasing TCE was observed at all monitoring locations within or downgradient of the biowall system. Concentrations of TCE continued to decrease even further from September to December 2005, and remained relatively stable from December 2005 to January 2006. In January 2006, concentrations of TCE have decreased to non-detect in the four monitoring wells located within the biowalls, and concentrations of TCE in the downgradient monitoring wells have been lowered to a range from 2.9 μg/L to 25 μg/L.

TABLE 2
SUMMARY OF VOLATILE ORGANIC COMPOUNDS IN GROUNDWATER

Sample Identification	Sample Date	PCE[a/] (µg/L)[b/]	TCE[a/] (µg/L)	1,1-DCE[a/] (µg/L)	*cis*-1,2-DCE (µg/L)	*trans*-1,2-DCE (µg/L)	VC[a/] (µg/L)	Acetone (µg/L)	2-Butanone (µg/L)	2-Hexanone (µg/L)
Downgradient Well										
PT-22	01-Dec-05	<1.0[c/]	46	<1.0	120	2.3	17	<5.0	<5.0	<5.0
	12-Dec-05	<1.0	42	<1.0	160 J[d/]	3.8	30	3.8 J	<5.0	<5.0
	24-Jan-06	<1.0	37	<1.0	110	2.6	26	<5.0	<5.0	<5.0
South Transect										
PT-12A	07-Sep-05	<50	860	<50	910	<50	<50	<50	<50	<50
(15' Upgradient)	24-Oct-05	<1.0	730	1.3	800	11	24	<5.0	<5.0	<5.0
	12-Dec-05	<1.0	385	0.55 J	315	4.9	8.2	<5.0	<5.0	<5.0
	24-Jan-06	<1.0	530	<1.0	400	5.6	19	<50	<50	13 J
MWT-18	07-Sep-05	<50	28 J	<50	120	<50	<50	1,200 J	2,500 J	27 J
(In East Biowall)	24-Oct-05	<20	<20	<20	190	<20	<20	3,000	4,400	<100
	12-Dec-05	<5.0	<5.0	<5.0	230	<5.0	23	4,700 J	7,600	49
	24-Jan-06	<20	<20	<20	150	<20	26	1,800	5,800	<100
MWT-19	07-Sep-05	<10	110	2.0 J	1,300	13	17	370	600	4 J
(Between biowalls)	24-Oct-05	<5.0	33	<5.0	1,600	21	18	190	200	<25
	12-Dec-05	<5.0	17	2.1 J	1,000	17	140 J	180	330	<25
	24-Jan-06	<1.0	22	1.4	870	20	345	170 J	455 J	5.7 J
MWT-20	07-Sep-05	<250	<250	<250	160 J	<250	<250	3,200	1,700	<250
(In West Biowall)	24-Oct-05	<5.0	<5.0	<5.0	160	2.9 J	16	270 J	990 J	34
	12-Dec-05	<5.0	<5.0	<5.0	13	2.2 J	13 J	200	260	<25
	24-Jan-06	<1.0	<1.0	<1.0	8.4	1.8	9.1	410 J	660	17 J
MWT-21	07-Sep-05	<100	98 J	<100	1,200	<100	<100	250	270	<100
(7.5' downgradient)	24-Oct-05	<1.0	45	2.4 J	1,400	38	69	350 J	310 J	6.0
	12-Dec-05	<5.0	20	<5.0	570	22	180	73	66	<25
	24-Jan-06	<1.0	18	0.74 J	470	20	180	130 J	110 J	<5.0
MWT-22	07-Sep-05	<100	<100	<100	1,000	<100	<100	400	480	<100
(22.5' downgradient)	24-Oct-05	<5.0	25	<5.0	1,100	17	170	340	310	<25
	12-Dec-05	<5.0	12	<5.0	360	11	140	66	89	<25
	24-Jan-06	<1.0	25	0.72 J	430	13	140	14 J	12 J	<5.0

(continued)

TABLE 2 (Continued)
SUMMARY OF VOLATILE ORGANIC COMPOUNDS IN GROUNDWATER

Sample Identification	Sample Date	PCE[a/] (µg/L)[b/]	TCE[a/] (µg/L)	1,1-DCE[a/] (µg/L)	*cis*-1,2-DCE (µg/L)	*trans*-1,2-DCE (µg/L)	VC[a/] (µg/L)	Acetone (µg/L)	2-Butanone (µg/L)	2-Hexanone (µg/L)
North Transect										
MWT-12R	07-Sep-05	<80	705	<80	965	<80	86	<80	<80	<80
(15' Upgradient)	24-Oct-05	<1.0	725	2.7	895	23	85	3.5 J	<5.0	<5.0
	12-Dec-05	<1.0	760	2.9	980	21	64	3.8 J	<5.0	<5.0
	24-Jan-06	<1.0	540	2.3	650	17	67	5.6 J	<5.0	<5.0
MWT-13	07-Sep-05	<250	<250	<250	320	<250	<250	1,600	2,700	<250
(In East Biowall)	24-Oct-05	<20	<20	<20	410	<20	<20	8,000	9,300	<100
	12-Dec-05	<10	<10	<10	220	<10	41	4,900	6,000	62
	24-Jan-06	<1.0	<1.0	<1.0	52	1.9	55	1,600	2,000	38 J
MWT-14	07-Sep-05	<50	170	<50	1,000	<50	<50	660	910	<50
(Between biowalls)	24-Oct-05	<10	<10	<10	1,600	22	10	2,800	2,900	<50
	12-Dec-05	<10	<10	<10	550	15	230	2,300	2,800	36 J
	24-Jan-06	<1.0	2	<1.0	140	11	340	770	930	17 J
MWT-15	07-Sep-05	<50	<50	<50	170	<50	<50	3,400	820	<50
(In West Biowall)	24-Oct-05	<20	<20	<20	140	<20	36	140	690	<100
	12-Dec-05	<5.0	<5.0	<5.0	15	2.6 J	10	130	140	<25
	24-Jan-06	<1.0	<1.0	<1.0	3.1	2.2	5.0	55 J	33 J	<5.0
MWT-16	07-Sep-05	<20	70	<20	160	<20	<20	270	120	<20
(7.5' downgradient)	24-Oct-05	<20	9.5 J	<20	380	<20	51	740	750	<100
	12-Dec-05	<5.0	2.5 J	<5.0	58	5.3	31	85	210	<25
	24-Jan-06	<1.0	2.9	<1.0	43	5.4	31	24 J	15 J	<5.0
MWT-17R	07-Sep-05	<10	33	<10	59	<10	<10	<10	<10	<10
(22.5' downgradient)	24-Oct-05	<1.0	16	<1.0	380	5.9	19	430 J	290 J	3.6 J
	12-Dec-05	<5.0	4.8 J	<5.0	120	4.4 J	42	79	180	<25
	24-Jan-06	<1.0	12	<1.0	97	4.2	60	11	6.2	<5.0

[a/] PCE = tetrachloroethene, TCE = trichloroethene, DCE = dichloroethene, VC = vinyl chloride.

[b/] µg/L = micrograms per liter.

[c/] <1.0 indicates the compound was not detected above the quantification limit indicated.

[d/] J-flag indicates the concentration is below the quantification limit but above the method detection limit, and the concentration is estimated.

Figure 4 and **Figure 5** show concentrations of TCE, *cis*-DCE, VC, and ethene along each well transect (oriented with the direction of groundwater flow) for the 6 week and 13 week sampling events. Total molar concentrations of chloroethenes are also plotted to show overall reductions in contaminant mass. In each event, a reduction in TCE is evident. Concentrations of *cis*-DCE are reduced within each biowall, but rebound sharply between the two biowalls, particularly during the 6 week monitoring event.

It is likely that at least a portion of the rebound in concentrations of *cis*-DCE between and downgradient of the biowalls is due to desorption of TCE and transformation to *cis*-DCE. Based on the fraction of organic carbon in the native sediments and the aqueous to organic carbon partitioning ratio of TCE, approximately 90% of the mass of TCE within the aquifer system is sorbed to the aquifer matrix. Decreasing the concentration of TCE and increasing the concentration of dissolved organic carbon in groundwater will lead to enhanced desorption of TCE from the aquifer matrix. Subsequent transformation to *cis*-DCE may cause concentrations of *cis*-DCE, which sorbs less strongly, to increase. This effect is greatly reduced during the 13 week sample event and should diminish over time. An apparent accumulation of *cis*-DCE in the South Transect suggest that this portion of the biowall system has not acclimated as rapidly as the North Transect.

Observing the relative concentrations of TCE and the by-products generated during reductive dechlorination, progression of the biodegradation process is evident within the Ash Landfill biowall system. The theoretical change in concentration over time or distance that is expected during sequential reductive dechlorination of chlorinated ethenes is shown on **Figure 6**, and outlined in the following steps:

1. TCE is the predominant contaminant source.
2. As TCE is reduced, DCE levels increase.
3. DCE decreases as TCE is depleted and DCE is converted to VC.
4. Finally, VC decreases as DCE is depleted and VC is converted to ethene.

Figure 7 shows the molar percent of total chlorinated ethenes (including ethene and ethane) as a function of distance along the biowall North Transect at 27 weeks after biowall installation. Reductive dechlorination has proceeded from Step 1 (TCE predominates) upgradient of the first biowall to Step 2 (conversion of TCE to DCE) within first (East) biowall. Following the path of groundwater flow along the monitoring transect, dechlorination has proceeded to Step 3 (conversion to VC) and Step 4 (conversion of VC to ethene) from the first to the second biowall.

In observing the data at 27 weeks after biowall installation, it is clear that adaptation and maturing of an anaerobic microbial population capable of complete conversion of TCE to ethene has occurred. The trends described above can also be shown on a point-by-point basis along both treatment transects. In observing the fraction of total ethenes over time at certain points within the North and South Transects, it is evident that the reaction zone within the South Transect is developing at a slower rate than in the North Transect.

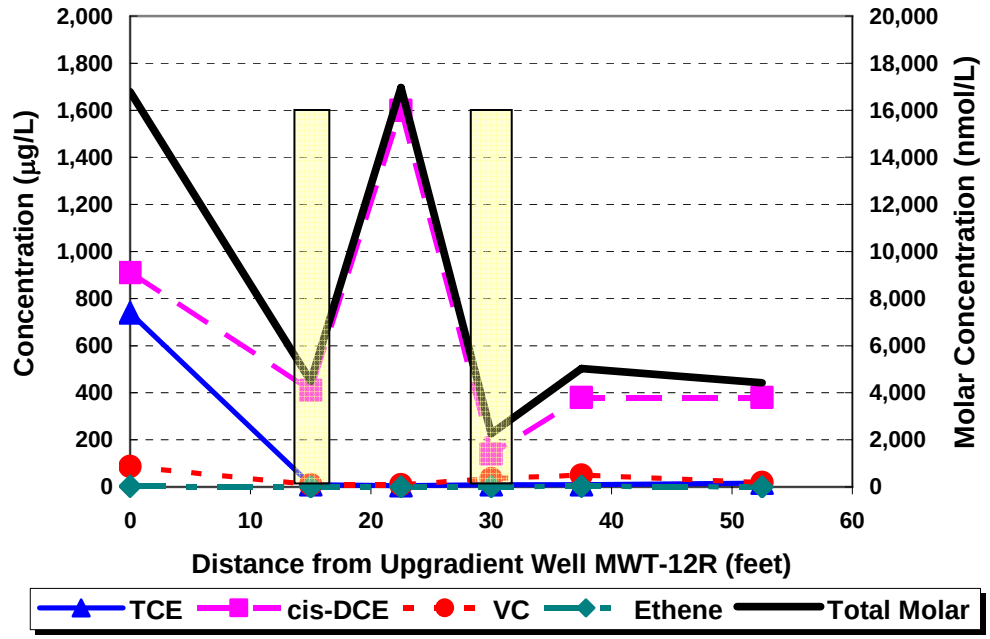

Figure 4A. Concentrations of Chloroethenes and Total Molar Chloroethenes Along the North Transect at 13 Weeks

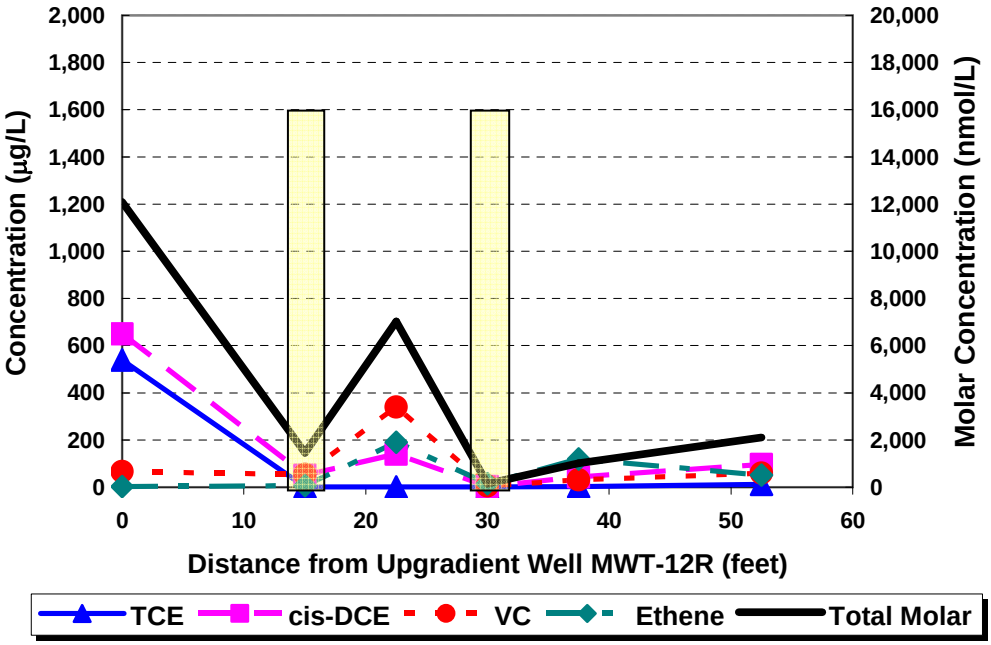

Figure 4B. Concentrations of Chloroethenes and Total Molar Chloroethenes Along the North Transect at 27 Weeks

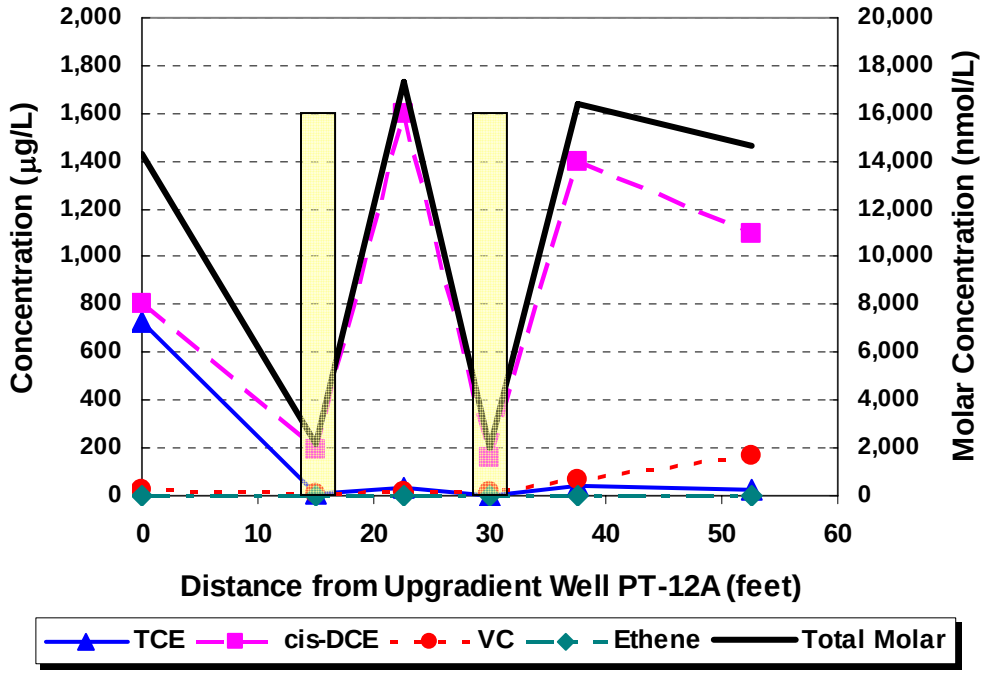

Figure 5A. **Concentrations of Chloroethenes and Total Molar Chloroethenes Along the South Transect at 13 Weeks**

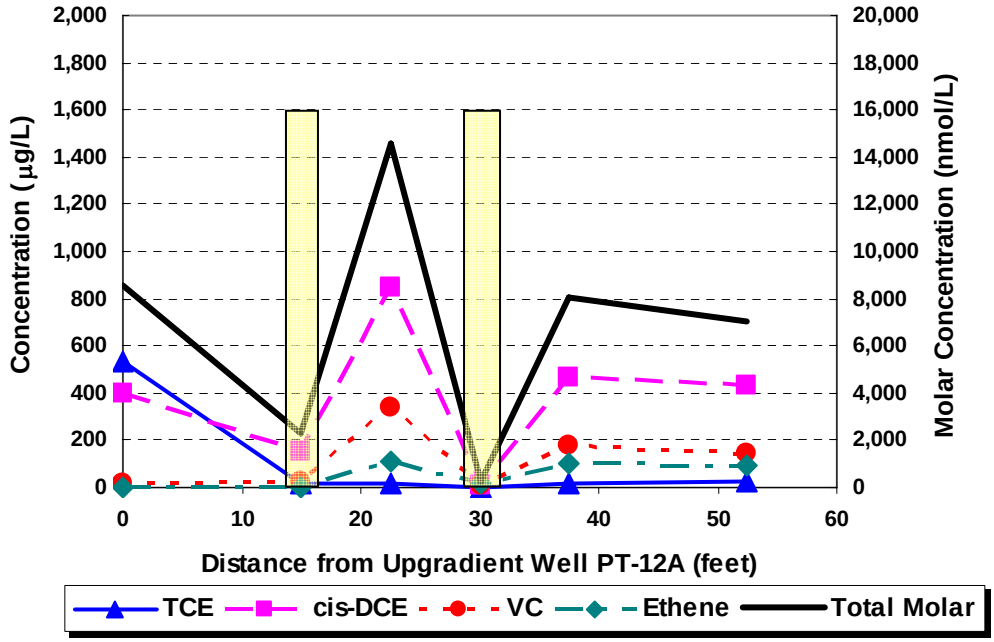

Figure 5B. **Concentrations of Chloroethenes and Total Molar Chloroethenes Along the South Transect at 27 Weeks**

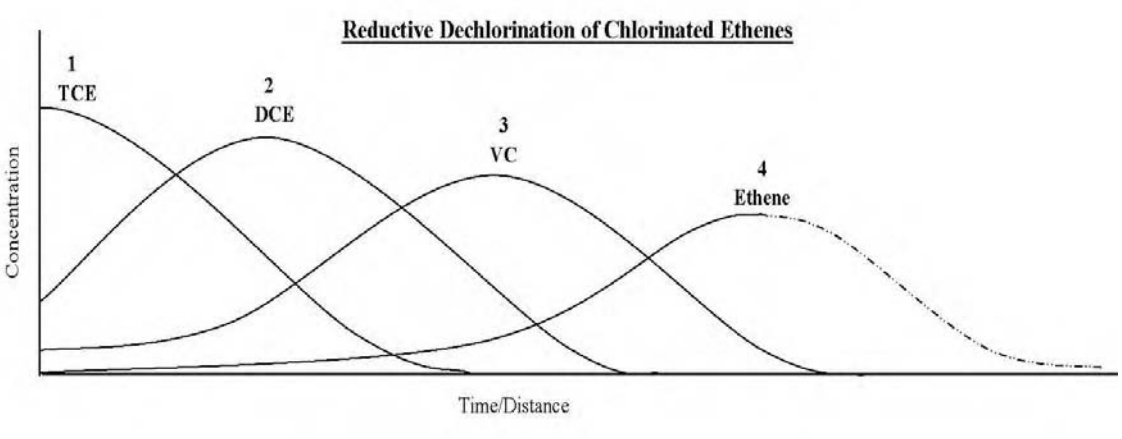

Figure 6. **Theoretical Changes in Molar Concentrations During Sequential Reductive Dechlorination**

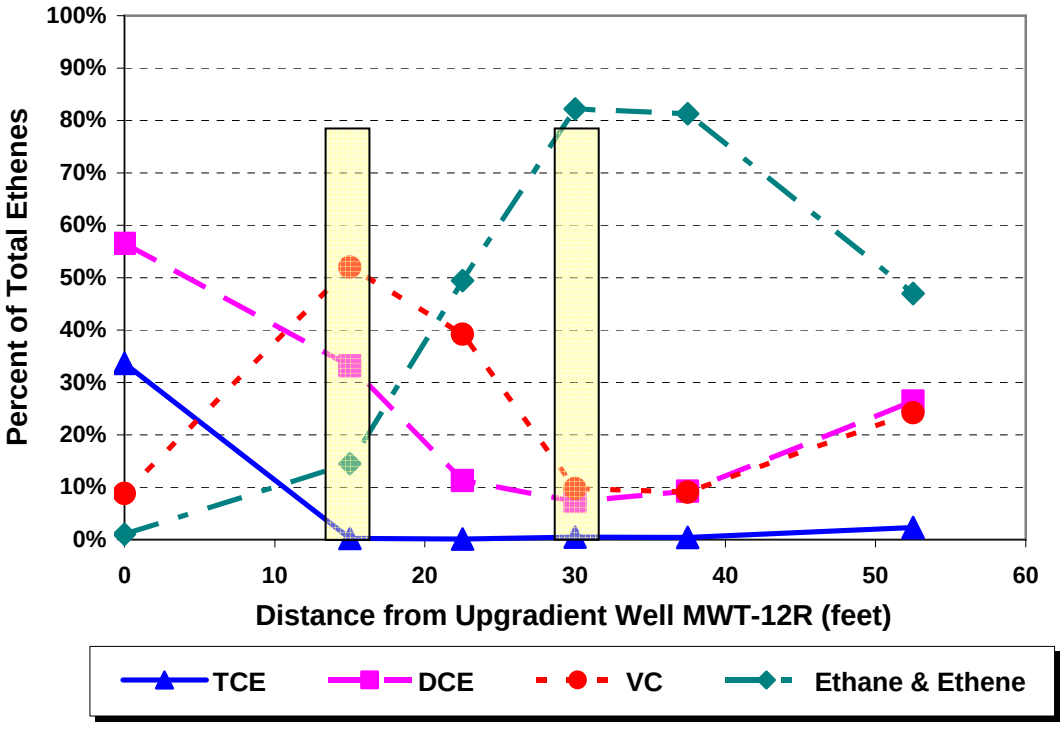

Figure 7. **Percent Molar Fractions for Chloroethenes and Ethene/Ethane for the North Transect at 13 Weeks**

4.4.2 Total Molar Concentrations of Chlorinated Ethenes

The total molar concentration of chlorinated ethenes within the second (West) biowall relative to the upgradient locations are shown in **Table 3**. The total molar concentrations are calculated by dividing the concentrations of PCE, TCE, DCE, and VC by their molecular weight and then summing the results. Percent reductions in total molar concentrations of chloroethenes over time along the North and South Transects have ranged from approximately 86 to 99 percent. A reduction in total molar concentrations shows that the chlorinated ethenes are not simply being converted from one chlorinated ethene to another (*i.e.*, accumulation of *cis*-DCE or VC), and that complete reduction to non-toxic degradation products (*e.g.*, ethene) is occurring.

Total molar concentrations of chloroethenes are clearly depleted within the biowalls. A decrease in total molar concentrations is observed along the North Transect both within and downgradient of the biowall. An increase in total molar concentration downgradient of the biowall along the South Transect (as shown in **Table 3**) may be 1) due to the continued desorption of TCE from native soils, 2) due to the mixing with untreated groundwater, or 3) indicate that biodegradation may be limited to the immediate biowall reactive zone at this time.

While the transformation of TCE to DCE may result in a temporary accumulation of *cis*-DCE in some locations, there remains a significant overall loss of chlorinated ethene mass (greater than 86 percent within the biowalls relative to upgradient locations).

4.4.3 Mass Flux and Estimate of Sorbed Mass

An evaluation of contaminant mass flux through the biowall system serves as a measure of system performance in reducing contaminant mass. Reduction in mass on a weight basis can be estimated by calculating the mass flux of soluble contaminant that enters the dual biowall system, and then comparing that to the mass flux of soluble contaminant exiting the second biowall (West Biowall). The mass flux was calculated (calculations not shown) using the concentration of each chlorinated ethene multiplied by the volume of water estimated to pass through the trench during a given time period. Based on these calculations, the mass reduction of chlorinated ethenes through the dual biowall system is between 98% for the South Transect to over 99% for the North Transect.

It should also be noted that a reduction in concentrations of TCE downgradient of the biowall would also result in desorption of TCE from the soil matrix. Based on the fraction of organic carbon in the aquifer matrix, ten times as much contaminant mass may be sorbed to the soil as is dissolved in the groundwater. It is likely that a portion of the rebound in concentrations of *cis*-DCE downgradient of the biowall is due to desorption of TCE, with subsequent transformation to *cis*-DCE. Because of the effects of desorption and mixing downgradient of the biowall trenches, the concentrations of chlorinated ethenes within the biowall (wells MWT-15 and MWT-20) are the most meaningful indicators of biowall performance.

TABLE 3

PERCENT REDUCTIONS OF TCE AND TOTAL CHLOROETHENES ALONG BIOWALL FLOWPATHS

North Transect									
Reductions in Concentration of TCE [a/]									
	Within Second Biowall			**Immediately (7.5 feet) Downgradient**			**Further (22.5 feet) Downgradient**		
Date	TCE MWT-12R (µg/L)[b/]	TCE MWT-15 (µg/L)	Percent Reduction TCE	TCE MWT-12R (µg/L)[b/]	TCE MWT-16 (µg/L)	Percent Reduction TCE	TCE MWT-12R (µg/L)[b/]	TCE MWT-17R (µg/L)	Percent Reduction TCE
September-05	705	<1.6	**99.9%**	705	70	**90.1%**	705	33	**95.3%**
October-05	725	<10	**99.3%**	725	9.5	**98.7%**	725	16	**97.8%**
December-05	760	<5	**99.7%**	760	<5	**99.7%**	760	4.8	**99.4%**
January-06	540	<1	**99.9%**	540	2.9	**99.5%**	540	12	**97.8%**
Reductions in Molar Concentration of Total Chloroethenes									
	Within Second Biowall			**Immediately (7.5 feet) Downgradient**			**Further (22.5 feet) Downgradient**		
Date	Total Molar Chlorethenes MWT-12R (nmol/L)[c/]	Total Molar Chlorethenes MWT-15 (nmol/L)c/	Percent Reduction Total Molar Chloroethenes	Total Molar Chlorethenes MWT-12R (nmol/L)[c/]	Total Molar Chlorethenes MWT-16 (nmol/L)	Percent Reduction Total Molar Chloroethenes	Total Molar Chlorethenes MWT-12R (nmol/L)[c/]	Total Molar Chlorethenes MWT-17R (nmol/L)	Percent Reduction Total Molar Chloroethenes
September-05	16,731	1,791	**89.3%**	16,731	2,196	**86.9%**	16,731	866	**94.8%**
October-05	16,190	2,192	**86.5%**	16,190	4,942	**69.5%**	16,190	4,411	**72.8%**
December-05	17,167	401	**97.7%**	17,167	1,209	**93.0%**	17,167	2,033	**88.2%**
January-06	12,089	147	**98.8%**	12,089	1,026	**91.5%**	12,089	2,103	**82.6%**
South Transectc									
Reductions in Concentration of TCE [a/]									
	Within Second Biowall			**Immediately (7.5 feet) Downgradient**			**Further (22.5 feet) Downgradient**		
Date	TCE PT-12A (µg/L)	TCE MWT-20 (µg/L)	Percent Reduction TCE	TCE PT-12A (µg/L)	TCE MWT-21 (µg/L)	Percent Reduction TCE	TCE PT-12A (µg/L)	TCE MWT-22 (µg/L)	Percent Reduction TCE
September-05	860	<8.1	**99.5%**	860	98	**88.6%**	860	<3.2	**99.8%**
October-05	730	<2.5	**99.8%**	730	45	**93.8%**	730	25	**96.6%**
December-05	400	<5	**99.4%**	385	20	**94.8%**	385	12	**96.9%**
January-06	530	<1	**99.9%**	530	18	**96.6%**	530	25	**95.3%**
Reductions in Molar Concentration of Total Chloroethenes									
	Within Second Biowall			**Immediately (7.5 feet) Downgradient**			**Further (22.5 feet) Downgradient**		
Date	Total Molar Chlorethenes PT-12A (nmol/L)[c/]	Total Molar Chlorethenes MWT-20 (nmol/L)c/	Percent Reduction Total Molar Chloroethenes	Total Molar Chlorethenes PT-12A (nmol/L)[c/]	Total Molar Chlorethenes MWT-21 (nmol/L)	Percent Reduction Total Molar Chloroethenes	Total Molar Chlorethenes PT-12A (nmol/L)[c/]	Total Molar Chlorethenes MWT-22 (nmol/L)	Percent Reduction Total Molar Chloroethenes
September-05	15,964	1,838	**88.5%**	15,964	13,187	**17.4%**	15,964	10,391	**34.9%**
October-05	14,321	1,966	**86.3%**	14,321	16,307	**-13.9%**	14,321	14,453	**-0.9%**
December-05	6,370	425	**93.3%**	6,370	9,180	**-44.1%**	6,370	6,199	**2.7%**
January-06	8,530	263	**96.9%**	8,530	8,082	**5.2%**	8,530	7,011	**17.8%**

[a/] TCE = trichloroethene [b/] µg/L = micrograms per liter. [c/] nmol/L = nanomoles per liter.

4.5 Other Compounds

Acetone, 2-butanone and 2-hexanone have also been detected in monitoring wells located within the biowalls, with concentrations up to 9,300 µg/L for 2-butanone at location MWT-13 in October 2005 (**Table 2**). These compounds, produced by fermentation reactions, are not anticipated to be stable outside of the highly reducing conditions established within the biowall trenches. Concentrations of these compounds decreased by over an order of magnitude (to 750 µg/L or less) in downgradient locations at 7.5 feet from the West Biowall. Furthermore, concentrations of these compounds were less than 14J µg/L (estimated concentration) at 22.5 feet downgradient of the biowalls in January 2006, and concentrations were non-detect at the furthest downgradient well (PT-22, 150 feet from the biowalls) in January 2006.

The concentrations of acetone, 2-butanone and 2-hexanone correlate to elevated concentrations of TOC and metabolic acids. The magnitude of the concentrations of acetone, 2-butanone, and 2-hexanone within the biowall anaerobic reaction zone decreased as the levels of TOC and metabolic acids decreased. Therefore, it is not anticipated that these compounds will adversely impact groundwater quality outside of the immediate biowall treatment zone.

5. PERFORMANCE ANALYSIS

Five performance objectives were developed (Section 1.1) to evaluate the effectiveness of the biowalls. The evaluation of these five objectives was used to justify the selection of mulch as the media for a full-scale system for the groundwater operable unit as required in the ROD for the Ash Landfill Site (Parsons, 2005). An assessment of these objectives are discussed below:

Objective 1: Achieve a similar or better reduction in concentrations of TCE within the biowall system relative to a ZVI wall previously installed downgradient of the mulch biowall pilot test.

***Assessment*:** Reductions in the concentrations of TCE are greater than 99% when comparing the upgradient wells to the wells within the West Biowall (**Table 3**). For comparison, the reductions in concentrations of TCE in the ZVI wall range from 94% to greater than 99% when evaluating data from 1999 and 2000, within the first year of operation (**Table 4**). The mulch biowall system has achieved results comparable to, or better than, performance of the ZVI wall in degrading TCE.

Objective 2: Demonstrate that the biowalls create a treatment zone within and downgradient of the trenches that is favorable to the long-term degradation of TCE, *cis*-DCE and VC.

Assessment: Geochemical parameter indicate that anaerobic zones within and downgradient of the biowalls have been established. Lowered DO, nitrate, and sulfate concentrations indicate that these electron receptors have been depleted. Increases in manganese, ferrous iron, and methane further indicate that highly reducing conditions have been induced. As described in Section 4.1, it is clear that complete reductive dechlorination has been stimulated. The production of ethene is a positive indication that complete dechlorination of the chlorinated ethenes present at the site. A dual biowall system is adequate to create a reaction zone sufficient to degrade *cis*-DCE and VC.

Objective 3: Demonstrate a reduction in total molar concentrations of CAHs in both the biowalls and at downgradient monitoring locations (i.e., complete degradation and not just transformation from one chlorinated compound to another).

Assessment: As shown in **Table 3**, the total molar chlorinated ethene reduction is between 86.3% and 98.8% when comparing the upgradient wells MWT-12R and PT-12A to the wells in the West Biowall (MWT-15 and MWT-20). During the last round of sampling, between 96.9% and 98.8% reduction in chlorinated ethenes was observed in both transects. For comparison, reductions in concentrations of total molar chlorinated ethenes in the ZVI wall during the first year of operation was between 69.5% and 99.4% (**Table 4**). The reduction in concentrations of total molar chloroethenes are equal to or greater in the West Biowall than in the ZVI wall.

Downgradient of the biowalls, the reduction in concentrations of total molar chlorinated ethenes varies as shown in **Table 3**. In the North Transect, reduction immediately downgradient in MWT-16 and further downgradient in MWT-17R ranged from 82.6% to 91.5% during the last round of sampling. In the South Transect, the percent reduction does not appear to reflect what is occurring within the West Biowall. During the last sampling round, the percent reduction of total chlorinated ethenes was between 5.2% and 17.8%. As discussed in **Section 4.1**, an increase in total molar concentration downgradient of the biowall within the South Transect may be due to 1) continued desorption of CAHs from downgradient soils, 2) mixing with untreated groundwater, or 3) indicate that biodegradation may be limited to the immediate biowall reactive zone at this time. While the transformation of TCE to *cis*-DCE may result in a temporal accumulation of *cis*-DCE in some locations, there remains a significant overall loss of chlorinated ethene mass (greater than 86 percent within the biowalls relative to upgradient locations).

Based on the data collected during the ZVI wall pilot study (1999/2000), reductions in total molar chlorinated ethenes downgradient of the ZVI wall (**Table 4**) ranged from 41.2% to 82.7% (2.5 feet from the wall). Using the most recent rounds of monitoring results at the ZVI wall (2004), reductions in total chlorinated ethenes ranged from -18.6% to 67.5%.

Variations between the distances at which the concentrations are measured downgradient (2.5 feet for the ZVI wall versus 7.5 to 22.5 feet downgradient for the biowalls), and the magnitude of concentrations within which each wall is located, may also affect the variability of the total molar chlorinated ethene reduction observed downgradient of these systems. A rebound effect may be observed as groundwater flows out of the reactive zone and begins to mix with residual contamination downgradient of the reactive wall. Residual levels are considerably lower near the ZVI wall than near the biowalls. However, the North Transect results in the West Biowall are considerably better than the ZVI wall.

Objective 4: Demonstrate that CAHs will not exceed NYSDEC GA Standards at a Farm House west of the site at any time during the estimated remediation timeframe.

Assessment: Sampling conducted in Round 2 included well MW-56, located approximately 1,250 feet upgradient of the Farm House. This well location has not been impacted by CAHs in groundwater. Monitoring required in the ROD will be conducted to ensure that groundwater in the vicinity of the Farm House remains unaffected.

TABLE 4
PERCENT REDUCTIONS IN TCE AND TOTAL CHLOROETHENES
IN THE ZERO-VALENT IRON WALL

1. Reductions in Concentration of TCE within the ZVI Wall [a/]

	North Transect			South Transect		
Date	TCE MWT-1 (µg/L)[b/]	TCE MWT-2 (µg/L)	Percent Reduction TCE	TCE MWT-7 (µg/L)	TCE MWT-8 (µg/L)	Percent Reduction TCE
TS Rounds						
April-99	23	1	**95.7%**	430	<1	**99.9%**
June-99	8	<1	**93.8%**	530	<2	**99.8%**
September-99	<2	<1	**N/A**	480	<1	**99.9%**
January-00	18	<2	**94%**	480	<3	**99.7%**
Latest Rounds						
March-04	17	3.2	**81.4%**	386	<0.5	**99.9%**
August-04	22	0.8	**96.4%**	280	1.8	**99.4%**

2. Reductions in Molar Concentration of Total Chloroethenes within the ZVI Wall

	North Transect			South Transect		
Date	Total Molar Chlorethenes MWT-1 (nmol/L)[c/]	Total Molar Chlorethenes MWT-2 (nmol/L)	Percent Reduction Total Molar Chloroethenes	Total Molar Chloroethenes MWT-7 (nmol/L)	Total Molar Chlorethenes MWT-8 (nmol/L)	Percent Reduction Total Molar Chloroethenes
TS Rounds						
April-99	981	299	**69.5%**	3,768	22	**99.4%**
June-99	417	79	**81.1%**	4,772	467	**90.2%**
September-99	81	21	**74.1%**	4,352	87	**98.0%**
January-00	924	267	**71.1%**	4,222	612	**85.5%**
Latest Rounds						
March-04	565	216	**61.8%**	3,159	898	**71.6%**
August-04	1,260	178	**85.9%**	2,463	1,593	**35.3%**

3. Reductions in Total Chloroethenes Downgradient of the ZVI Wall

	North Transect			South Transect		
Date	Total Molar Chlorethenes MWT-1 (nmol/L)	Total Molar Chlorethenes MWT-3 (nmol/L)	Percent Reduction Total Molar Chloroethenes	Total Molar Chloroethenes MWT-7 (nmol/L)	Total Molar Chlorethenes MWT-9 (nmol/L)	Percent Reduction Total Molar Chloroethenes
TS Rounds						
April-99	981	312	**68.2%**	3,768	684	**81.8%**
June-99	417	122	**70.7%**	4,772	2,048	**57.1%**
September-99	81	35	**56.8%**	4,352	862	**80.2%**
January-00	924	543	**41.2%**	4,222	730	**82.7%**
Latest Rounds						
March-04	565	307	**45.7%**	3,159	1,506	**52.3%**
August-04	1,260	410	**67.5%**	2,463	2,922	**-18.6%**

[a/] TCE = trichloroethene [b/] µg/L = micrograms per liter. [c/] nmol/L = nanomoles per liter.

Objective 5: Evaluate biowall design criteria (*e.g.*, generation of organic carbon, degradation rates, residence time) and constructability issues (*e.g.*, trenching techniques, vegetable oil application, and subsurface pipe placement) required for effective long-term operation.

Assessment: Sufficient data has been collected during the pilot study to make a reasonable assessment for implementing a full-scale biowall system. Biowall trenches may be installed using a conventional backhoe equipped with hard teeth on the excavator bucket. A dual biowall system that creates a continuous reaction zone between the two biowalls is required to achieve complete dechlorination to ethene. The backfill material is sufficient to induce anaerobic conditions, and coating the mulch in the upgradient biowall with vegetable oil provides a useful amendment for bioavailable organic carbon.

6. SUMMARY AND PATH FORWARD

Results of the Ash Landfill biowall pilot test are summarized below:

- Reductions in the concentration of TCE between the upgradient wells and the wells within the second biowall (West Biowall) are greater than 99%.
- Reductions in total molar chlorinated ethenes between the upgradient wells and the wells within the second biowall (West Biowall) are between 86% and 99%.
- Geochemical data and reductions in chlorinated ethenes indicates that treatment zones have been readily established within and downgradient of the dual biowall system. Development of the treatment zone along the South Transect, although present, appears to lag development along the North Transect by about 40 to 50 days.
- The molar fraction of ethene is increasing within and downgradient of the biowall system and is a positive indicator of complete dechlorination of chlorinated ethenes at the site.
- Sufficient performance relative to the ZVI wall and sufficient design information has been acquired during the pilot test to design and install a full-scale system.

The biowall performance has been shown to be comparable to, and in some cases better than, that of the ZVI wall during its first year of performance. Based on the performance and cost of the pilot biowall system relative to the pilot-scale ZVI wall, a full-scale biowall system has been selected as the final remedy for the Ash Landfill site to prevent off-Depot migration of CAHs and to reduce the overall time for site cleanup.

The final full-scale design utilizes three sets of dual biowalls along the axis of the plume to reduce the overall time frame for remediation of the CAH plume. Approximately 2,720 linear feet of biowalls has been installed, using approximately 6,240 cubic yards of a 50:50 mulch to sand mixture coated with approximately 15,600 gallons of vegetable oil. The full-scale system is currently being monitored on a quarterly basis.

7. ACKNOWLEDGEMENTS

Parsons Infrastructure & Technology Group, Inc. (Parsons) completed this project for the United States Army Corps of Engineers (USACE) under contract to the Air Force Center for Environmental Excellence (AFCEE). The authors specifically acknowledge the cooperation and assistance of the US Army.

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APPENDIX F.2

PERMEABLE MULCH BIOWALL AT LANDFILL 3, OPERABLE UNIT 1, ALTUS AIR FORCE BASE, OKLAHOMA

PERMEABLE MULCH BIOWALL AT LANDFILL 3, OPERABLE UNIT 1, ALTUS AIR FORCE BASE, OKLAHOMA

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1. INTRODUCTION

A permeable mulch biowall was installed in June 2002 at Landfill 3 (LF-03), Operable Unit 1 (OU-1), Altus Air Force Base (AFB), Oklahoma, as a demonstration of enhanced *in-situ* bioremediation of chlorinated solvents. The study was conducted by Parsons Infrastructure & Technology Group, Inc. (Parsons) for the Air Force Center for Engineering and the Environment (AFCEE). Additional data was provided by the United States Environmental Protection Agency (USEPA), National Risk Management Research Laboratory (NRMRL), Ground Water and Ecosystem Restoration Division (GWERD) for sample events in July 2004 and April 2007.

1.1 Remedial Objective

The objective of the biowall application is to contain and attenuate a shallow groundwater plume contaminated with trichloroethene (TCE) and *cis*-1,2-dichloroethene (*cis*-DCE) to prevent surface water discharge or off-base migration (**Figure 1**). In particular, the biowall was installed across the path of groundwater flow along the downgradient (eastern) edge of LF-03 to assess the feasibility of promoting the *in-situ* anaerobic bioremediation of TCE and *cis*-DCE in groundwater (**Figure 2**). The biowall is intended to capture over 80 percent of the mass discharge of chlorinated aliphatic hydrocarbons (CAHs, or chlorinated solvents) originating from the landfill.

1.2 Scope of Work

Site-specific activities conducted at LF-03 in support of the field demonstration (Parsons, 2002) included the following:

- Installation from 19 to 23 June 2002, of a 455-foot long, by 24-foot deep, by 1.5-foot wide mulch biowall composed of shredded bark mulch, cotton gin compost, and sand;
- Installation of 10 groundwater performance monitoring wells from 16 to 19 July 2002; and
- Post-installation sampling of the performance monitoring wells and existing monitoring wells OU-1-01 and WL019 in July 2002, September 2002, March 2003, November 2003, July 2004 (USEPA), and April 2005.

Groundwater samples were collected after installation of the biowall and were analyzed for CAHs, dissolved oxygen (DO), nitrate, nitrite, ferrous iron, manganese, sulfate, hydrogen sulfide, carbon dioxide, methane, ethane, ethane, oxidation-reduction potential (ORP), alkalinity, pH, temperature, specific conductance, total organic carbon (TOC), volatile fatty acids (VFAs), and chloride.

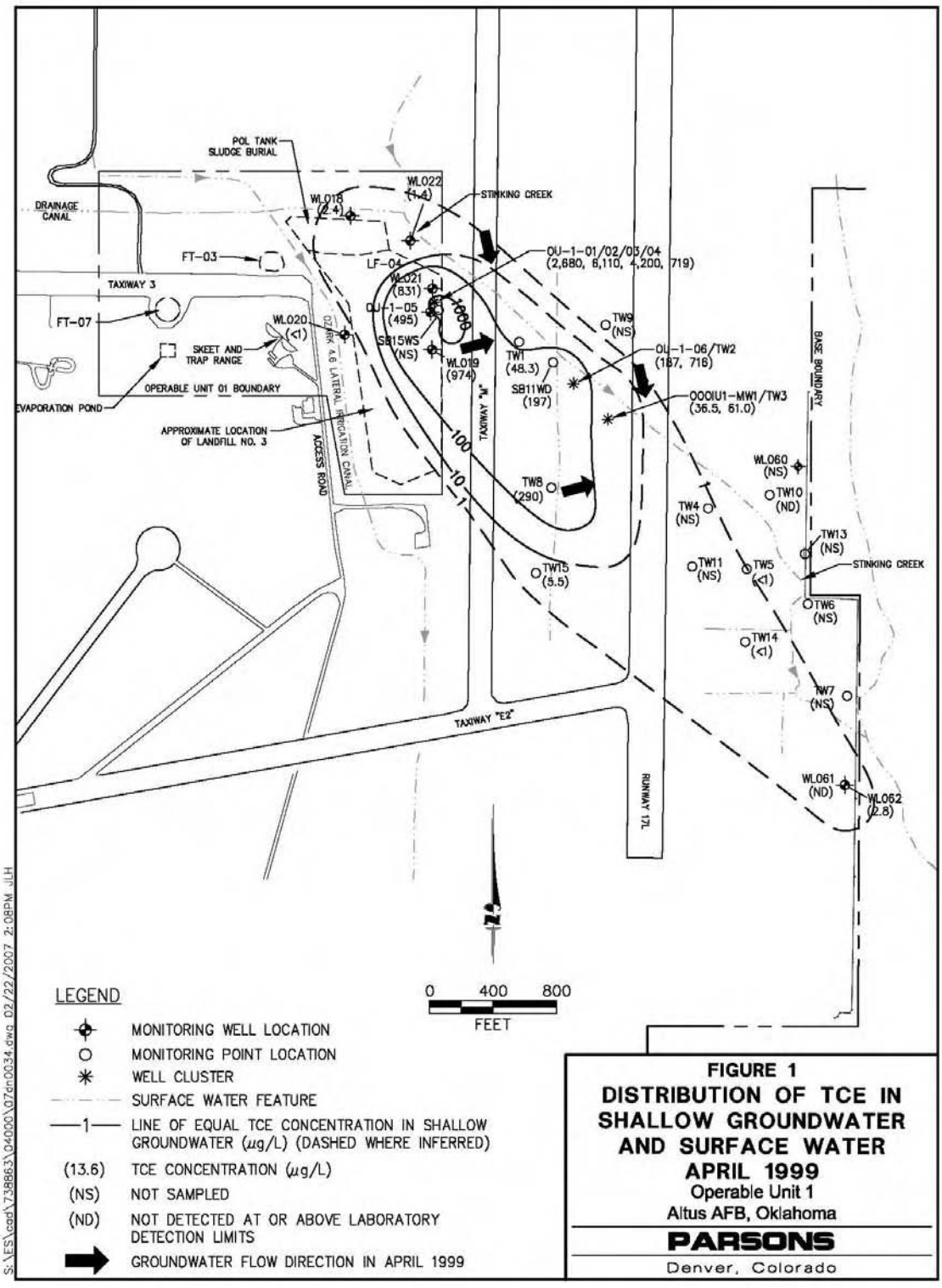

FIGURE 1
DISTRIBUTION OF TCE IN SHALLOW GROUNDWATER AND SURFACE WATER APRIL 1999
Operable Unit 1
Altus AFB, Oklahoma
PARSONS
Denver, Colorado

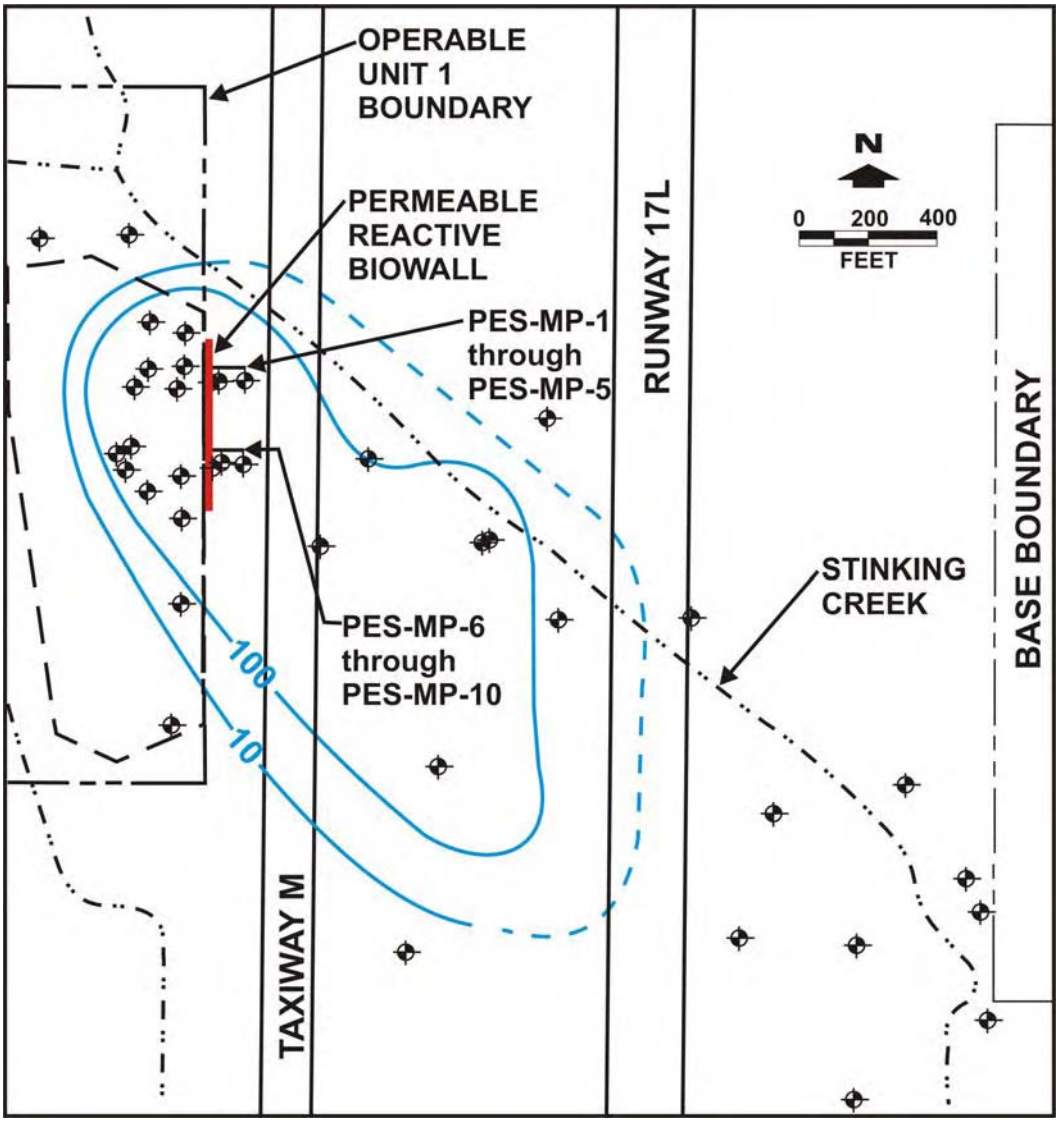

Figure 2. Location of Biowall Relative to TCE Plume (isoconcentration contours in micrograms per liter of TCE in April 1999)

The biowall was also sampled by the USEPA NRMRL/GWERD in July 2004. Groundwater samples were collected and analyzed for CAHs, DO, nitrate+nitrite (as nitrogen), ferrous iron, sulfate, hydrogen sulfide, methane, ethane, ethene, acetylene, ORP, alkalinity, pH, temperature, specific conductance, TOC, dissolved inorganic carbon (DIC), and chloride. The USEPA NRMRL/GWERD also installed an extensive network of 1-inch monitoring wells upgradient, within, and downgradient of the biowall. Data from a round of sampling in April 2007 was provided to AFCEE. This document utilizes select data from April 2007 for monitoring wells along two well transects that have been sampled since biowall installation. This provides additional evaluation of long-term biowall performance to approximately 58 months post biowall installation.

2. SITE DESCRIPTION

LF-03 is located at the eastern portion of OU-1, and is bordered by the Ozark lateral irrigation canal on the west and south, Stinking Creek on the northeast, an unnamed drainage canal on the north, and the Base boundary and Taxiway "M" on the east (**Figure 1**). From 1956 to 1965, LF-03 received waste materials including garbage, wood, paper, metal, and shop wastes. After 1965, LF-03 received construction debris, concrete, brush, and several drums of paint waste. Waste at LF-03 was buried in trenches at depths ranging from 6 to 8 feet below ground surface (bgs). Historical waste management activities at LF-03 have resulted in milligram per liter concentrations of CAHs in groundwater beneath and downgradient to the east-southeast of the landfill.

Surface soils at the site consist of approximately 5 feet of clayey silt and a weathered and fractured stiff silty clay that extends to depth of approximately 25 to 35 feet bgs. These sediments are underlain by cemented silt and dense shale of the Hennessey Group of Permian age. Shallow groundwater occurs under unconfined conditions and generally flows towards the east-southeast and Stinking Creek. Shallow groundwater at the site occurs at a seasonally variable depth of approximately 6 to 12 feet bgs.

The groundwater surface slopes toward the southeast with an average horizontal hydraulic gradient of approximately 0.003 foot per foot (ft/ft). An average hydraulic conductivity estimated from slug test data is approximately 8.7 feet per day (ft/day) in the overburden silty clay (Parsons, 2007). Using an estimated hydraulic conductivity of 8.7 ft/day, a horizontal hydraulic gradient of 0.003 ft/ft, and an estimated effective porosity of 15 percent, the advective groundwater flow velocity in the overburden silty clay is approximately 0.174 ft/day, or 64 feet per year (ft/yr). These are only average flow rates, actual flow rates may vary due to aquifer heterogeneity. In particular, visual examination of sediments from borehole cores indicates the presence of secondary permeability due to dissolution features and soil fractures. Groundwater flow through these high permeability zones may be an order of magnitude or more higher than the average rate estimated above.

TCE and the dichloroethene isomer *cis*-DCE are the most prevalent CAHs in both extent and concentration in groundwater at LF-03. The areal extent of TCE based on groundwater samples collected in April 1999 is illustrated in **Figure 1**. The TCE plume originates from LF-3 and extends southeastward approximately 4,000 feet to the eastern Base boundary. Concentrations of TCE measured in April 1999 ranged up to 6,110 micrograms per liter (µg/L).

Migration of the TCE plume to the east appears to be limited by Stinking Creek. Groundwater samples collected from monitoring locations northeast of Stinking Creek during previous investigations did not contain detectable levels of TCE or other CAHs (Parsons, 1999). Stinking Creek may be exerting hydraulic control, resulting in no further TCE plume migration northeast of the creek. Hydraulic control could occur under both gaining and losing stream scenarios, and could vary seasonally. Under a losing stream scenario, groundwater recharge could create a barrier to flow in the form of a groundwater divide. Under a gaining stream scenario, a significant percentage of under flow could be captured by the creek.

3. BIOWALL DESIGN AND INSTALLATION

A 455-foot long, by 24-foot deep, by 1.5-foot wide mulch biowall was installed from 19 to 23 June 2002 by DeWind One-Pass Trenching of Zeeland, Michigan (**Figure 3**). Final biowall composition consisted of approximately 300 cubic yards of shredded mulch, 60 cubic yards of cotton gin compost, and 265 cubic yards of sand. The mulch consisted of shredded plant material (a mix of deciduous and evergreen trees and shrubs) generated by the City of Altus after a winter storm event and during seasonal landscaping operations throughout the surrounding community.

Figure 3. LF-03 Biowall Installation Using a Continuous One-pass Trencher

A continuous trenching machine (**Figure 3**) was employed to excavate the trench for the biowall and simultaneously place the mulch, compost, and sand mixture into the trench. The trencher is a track-mounted vehicle that has a cutting boom resembling a large chain saw (*i.e.*, linked chain belt with cutting teeth). A steel box with a hopper assembly is fitted atop the cutting boom. The cutting boom excavates a trench by simultaneously rotating the cutting chain and advancing the boom until the desired depth of excavation has been achieved.

The steel box and hopper assembly provide for stabilization of the trench sidewalls during excavation and subsequent placement of the sand and mulch mixture, which is introduced through the feed hopper using a front end loader. Simultaneous excavation and placement of backfill materials eliminates concerns associated with open excavations. Soil generated during excavation of the biowall was graded atop the biowall. The location and extent of the biowall was marked with metal fence posts painted a high visibility color.

Following construction of the biowall, 10 groundwater monitoring wells were installed along two transects oriented perpendicular to the biowall. Wells were installed within the footprint of the biowall, and at distances of 5, 10, 30, and 100 feet downgradient (to the east) of the biowall. These points are used to monitor groundwater geochemical indicator parameters and contaminant concentrations within and immediately downgradient of the biowall. Two existing groundwater monitoring wells located approximately 25 feet upgradient of the biowall (OU-1-01 and WL019) were also monitored for background conditions.

4. PERFORMANCE MONITORING RESULTS

Monitoring results over a period of 58 months from July 2002 through April 2007 are presented in the following subsections on hydrogeology, groundwater geochemistry, substrate and electron donor distribution, and degradation of chlorinated ethenes. Two transects of monitoring wells are located along the approximate path of groundwater flow, perpendicular to the biowall trench (**Figure 2**). The northern transect consists of wells OU-1-01 and MP01 through MP05. The southern transect consists of wells WL019 and MP06 through MP10. Monitoring points MP01 and MP06 are located within the biowall trench.

4.1 Hydrogeology

Depth to groundwater within the biowall ranges from approximately 4.7 to 6.2 feet bgs at MP01, and from approximately 6.6 to 8.0 feet bgs at MP06. The depth of the trench is approximately 24 to 25 bgs. Therefore, the saturated thickness within the biowall trench may range from 16 to 19 feet at any given time, depending on seasonal changes in groundwater levels due to recharge from precipitation or from upgradient surface water canals.

As described in Section 2, weathered and fractured silty clay and silt extends to depth of approximately 25 to 35 feet bgs at the site, which is underlain by well-cemented silt and dense shale of the Hennessey Group. The biowall trench does not extend all the way to the low permeability sediments of the Hennessey Group, and some migration of contaminated groundwater underneath the biowall trench is anticipated to occur. In addition, the biowall trench does not intercept the entire width of the CAH groundwater plume. Therefore, mixing of treated groundwater from the biowall and contaminated groundwater downgradient of the biowall trench will occur to some degree. Monitoring results for well locations more than 10 feet downgradient of the biowall should be evaluated with the understanding that not all of the water at those monitoring locations may have passed through the biowall. Results for wells MP-01 and MP-06, located within the biowall trench, are the most representative of the degree to which the biowall is effective in degrading CAHs in groundwater passing through the biowall.

The residence time of groundwater and contaminants within the reactive biowall is useful when interpreting the extent and rate of anaerobic degradation that is occurring. The average rates of groundwater flow estimated in Section 2 is 0.174 ft/day using an estimated effective porosity of 15 percent. The biowall has a higher effective porosity (estimated to be 25 percent based on column studies by Shen and Wilson, 2007) due to the presence of large amounts of coarse sand. Because the biowall is of limited thickness, it can be assumed that the volumetric flow rate through the biowall is the same as through the aquifer formation. Therefore, the rate of groundwater flow in the biowall is estimated to be 0.10 ft/day. Using this rate and a biowall trench width of 1.5 feet, the average residence time of groundwater within the biowall is estimated to be 15 days, assuming groundwater flow is directly perpendicular to the biowall trench.

Groundwater residence time could be longer if there is a component of groundwater flow parallel to the biowall trench. Conversely, residence time could be considerably less where groundwater flows into and out of the trench along zones of high secondary permeability. Overall residence for CAHs will be greater than groundwater residence time due to the retarding effects of sorption to the biowall matrix.

4.2 Groundwater Biogeochemistry

Biodegradation causes measurable changes in groundwater geochemistry that can be used to evaluate the effectiveness of substrate addition in stimulating biodegradation. For anaerobic reductive dechlorination to be an efficient process, the groundwater typically must be sulfate-reducing or methanogenic. Thus, groundwater in which anaerobic reductive dechlorination is occurring should have the following geochemical signature:

- Depleted concentrations of DO, nitrate, and sulfate;
- Elevated concentrations of ferrous iron, manganese, methane, carbon dioxide, and alkalinity; and
- Reduced ORP.

Select geochemical parameters are shown on **Table 1**. Comparison of geochemical parameters for biowall locations MP01 and MP06 to locations outside the biowall are summarized below.

Dissolved Oxygen. With the exception of the furthest downgradient well locations, concentrations of DO were already depleted (less than 2.0 milligrams per liter [mg/L]) in the study area. As of April 2005, concentrations of DO were less than 1.0 mg/L at all sample locations, except OU-1-04 which purged dry. Measurements of DO at select wells (OU-1-1, MP01, MP04, MP06, and MP09) in April 2007 appear to be elevated relative to previous measurements at concentrations ranging from 1.57 to 2.47 mg/L. However, this data appears inconsistent with ORP data and may be an artifact of sampling method.

Oxidation-Reduction Potential. ORP was measured and reported in the field using meter readings referenced against a silver/silver chloride (Ag/AgCl) electrode. ORP upgradient of the biowall has ranged from -132 to 150 millivolts (mV). This indicates background conditions are mildly anoxic. For monitoring location OU-1-01, ORP was lowered from 37 mV in November 2003 to -132 mV in July 2004. This decrease in ORP may be due to the installation of a bioreactor upgradient of the biowall in the LF-03 source area in November 2003 (**Appendix F.3**).

Within the biowall (wells MP01 and MP06), ORP has been lowered to a range of –212 mV to
–365 mV during post-installation monitoring events. As of April 2007, ORP within the biowall was -270 mV at MP01 and -292 mV at MP06. These levels of ORP indicate conditions are sufficiently reducing within the biowall to support iron reduction, sulfate reduction, and methanogenesis. As of July 2004, all monitoring locations downgradient (to a distance of 100 feet) of the biowall exhibited ORP levels less than -80 mV, indicating that anaerobic conditions are present over a large area downgradient of the biowall as well. In April 2005, ORP at the furthest downgradient locations (100 feet from the biowall) rebounded to 25 mV at MP05 and 61 mV at MP10.

TABLE 1
GROUNDWATER GEOCHEMICAL DATA

Sample Location (feet from trench)	Sample Date	pH (su)[a/]	Dissolved Oxygen (mg/L)[b/]	Redox Potential (mV)[c/]	Total Organic Carbon (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Hydrogen Sulfide (mg/L)	Alkalinity (mg/L)	Dissolved Hydrogen (nM)[d/]	Methane (µg/L)[e/]	Ethane (µg/L)	Ethene (µg/L)
Northern Flow Path													
OU-1-01	17-Jul-02	6.79	<0.01	88	<5.0	<0.01	1,600	<0.01	380	NA[f/]	2.4	0.022	0.077
	18-Sep-02	6.90	<0.01	9	5.6	<0.01	1,700	0.10	340	NA	5.2	0.099	0.17
	20-Mar-03	7.03	1.93	41	6.4	<0.01	1,600	<0.01	272	NA	6.4	0.018	0.063
	11-Nov-03	6.70	0.63	37	4.5J	0.13	1,700	0.70	357	NA	17	0.11	0.540
(USEPA)	14-Jul-04	6.76	0.36	-132	5.2	<0.2	1,250	<0.10	290	NA	1.6	<2.0	<2.7
OU-1-04	19-Apr-05	6.75	8.2 [g/]	-90	12	0.16	1,600	0.25	308	NA	13	0.055	0.071
(USEPA)	11-Apr-07	6.76	1.75	-123	5.1	0.25	1,890	0.16	380	NA	31	<1.9	<2.6
MP01	18-Jul-02	6.75	0.09	-365	2,800	3.5	410	15	3,360	NA	8,800	0.008	0.065
	18-Sep-02	7.08	<0.01	-212	380	1.2	17	10	3,400	2.0	7,000	0.014	0.010
	20-Mar-03	6.82	1.67	-218	200	2.3	16	3.5	1,904	2.4	8,000	0.040	0.098
	12-Nov-03	6.54	0.43	-232	78	6.0	21	12	1,428	1.8	4,500	<0.005	0.012
(USEPA)	14-Jul-04	6.26	0.23	-222	28	3.4	9.5	1.4	850	NA	8,350	<2.0	<2.7
	19-Apr-05	6.23	0.60	-332	79	0.14	190	0.16	1,512	NA	12,000	0.006	3.3
(USEPA)	11-Apr-07	6.31	2.47	-270	7.9	0.45	245	66	1,830	NA	786	<1.9	<2.6
MP02	18-Jul-02	7.11	0.19	-94	19	0.68	1,900	2.4	300	NA	150	0.016	0.062
	18-Sep-02	6.82	<0.1	-179	43	2.3	1,700	3.2	900	1.7	3,500	0.011	0.22
	20-Mar-03	6.80	1.74	-158	24	2.9	1,700	0.60	306	0.60	1,800	0.008	0.18
	12-Nov-03	6.35	0.76	-116	25	3.0	1,600	0.90	816	1.3	3,700	0.052	0.095
(USEPA)	14-Jul-04	6.34	0.39	-135	47	3.4	1,560	<0.10	1,630	NA	6,030	<2.0	<2.7
	19-Apr-05	6.30	0.40	-340	100	0.07	930	0.72	1,780	NA	14,000	0.005	1.6
MP03	18-Jul-02	7.05	1.91	20	5.2	0.16	1,900	<0.01	260	NA	200	0.013	0.036
	18-Sep-02	6.67	<0.01	-68	16	0.21	1,900	80	--	NA	1,400	<0.005	0.100
	20-Mar-03	6.86	1.29	-70	15	0.40	2,000	1.0	340	NA	1,900	<0.005	0.079
	12-Nov-03	6.36	0.60	-148	15	1.8	2,000	0.70	714	NA	5,400	0.027	0.15
(USEPA)	14-Jul-04	6.32	0.23	-203	19	2.5	1,280	0.12	1,420	NA	6,740	<2.0	<2.7
	19-Apr-05	6.36	0.60	-206	36	1.0	1,100	0.23	984	NA	14,000	<0.005	0.800
MP04	18-Jul-02	6.62	0.01	-204	130	0.96	1,800	3.5	460	NA	1,900	0.039	0.360
	18-Sep-02	6.76	<0.01	-169	30	1.6	1,700	1.2	560	NA	4,100	<0.005	0.130
	20-Mar-03	6.75	1.25	-171	27	2.2	1,600	1.3	765	NA	5,600	<0.005	0.120
	11-Nov-03	6.38	0.64	-120	11	1.3	1,200	2.2	680	NA	6,500	0.034	0.120
(USEPA)	14-Jul-04	6.51	0.41	-112	10	1.5	1,180	<0.10	1,170	NA	6,830	<2.0	<2.7
	19-Apr-05	6.35	0.5	-188	28	1.4	1,300	0.05	836	NA	8,200	<0.005	0.410
(USEPA)	11-Apr-07	6.37	2.37	-117	7.7	2.6	1,840	0.04	960	NA	37	<1.9	<2.6
MP05	18-Jul-02	6.93	4.55	63	<5.0	<0.01	1,900	0.10	300	NA	4.8	0.023	0.032
	19-Sep-02	7.01	<0.01	26	5.4	<0.01	1,800	0.20	200	NA	14	0.052	0.110
	19-Mar-03	6.77	1.30	42	10	<0.01	1,700	0.20	340	NA	1,300	0.039	0.130
	10-Nov-03	6.52	0.48	-82	4.7J	0.13	1,800	0.20	408	NA	1,800	0.100	0.130
(USEPA)	14-Jul-04	6.48	0.45	-84	4.2	0.30	1,260	<0.10	770	NA	1,350	<2.0	<2.7
	20-Apr-05	6.57	0.50	25	10	0.11	1,100	<0.10	560	NA	1,700	<0.005	0.071

(continued)

TABLE 1 (concluded)
GROUNDWATER GEOCHEMICAL DATA

Sample Location (feet from trench)	Sample Date	pH (su)[a/]	Dissolved Oxygen (mg/L)[b/]	Redox Potential (mV)[c/]	Total Organic Carbon (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Hydrogen Sulfide (mg/L)	Alkalinity (mg/L)	Dissolved Hydrogen (nM)[d/]	Methane (µg/L)[e/]	Ethane (µg/L)	Ethene (µg/L)
Southern Flow Path													
WL019	17-Jul-02	6.90	<0.01	107	<5.0	<0.01	2,000	0.10	320	NA	4.2	0.029	<0.005
	19-Sep-02	6.92	<0.01	150	<5.0	<0.01	1,600	0.04	400	NA	3.6	0.042	0.045
	19-Mar-03	6.85	1.45	60	<5.0	<0.01	2,000	0.20	204	NA	26	0.014	0.011
	11-Nov-03	6.79	0.64	13	3.5J	<0.01	2,500	<0.03	238	NA	96	0.044	0.014
(USEPA)	14-Jul-04	6.79	0.45	111	1.3	<0.2	1,810	<0.10	280	NA	34	<2.0	<2.7
	18-Apr-05	6.72	0.60	94	10	0.04	1,900	0.03	394	NA	20	0.032	0.036
MP06	18-Jul-02	6.43	<0.01	-266	--	4.1	NA	40	2,400	NA	7,900	0.064	0.220
	17-Sep-02	6.98	<0.01	-325	390	0.3	300	14	3,720	2.2	7,900	0.064	0.063
	18-Mar-03	6.80	1.84	-342	140	0.12	350	94	3,400	1.3	8,500	0.008	0.11
	11-Nov-03	6.58	0.40	-364	82	0.46	340	273	2,448	1.5	5,300	<0.005	0.049
(USEPA)	14-Jul-04	6.38	0.18	-321	22	<0.2	903	0.72	1,390	NA	8,790	<2.0	<2.7
	18-Apr-05	6.34	0.60	-315	25	0.04	2200	0.10	685	NA	13,000	<0.005	0.27
(USEPA)	11-Apr-07	6.48	2.09	-292	14	0.15	625	75	1,900	NA	3,010	<1.9	<2.6
MP07	19-Jul-02	6.53	<0.01	-227	710	1.7	1,100	19	1,200	NA	2,500	0.100	0.790
	17-Sep-02	6.82	<0.01	-201	80	0.9	700	8.8	1,680	2.0	6,400	0.024	0.210
	18-Mar-03	6.49	1.25	-173	110	2.7	2,600	1.9	1,428	0.79	7,400	0.005	0.160
	11-Nov-03	6.43	0.63	-140	44	2.7	960	0.90	1,428	1.4	5,300	<0.005	0.160
(USEPA)	14-Jul-04	6.36	0.35	-218	65	2.4	1,080	<0.10	1,810	NA	5,310	<2.0	<2.7
	18-Apr-05	6.45	0.40	-152	51	2.1	1,300	0.22	1,492	NA	13,000	0.031	0.340
MP08	19-Jul-02	6.67	<0.01	-235	520	0.75	1,400	16	960	NA	1,700	0.300	0.970
	19-Sep-02	6.80	<0.01	-237	77	1.1	800	19	1,400	NA	6,200	0.030	0.240
	18-Mar-03	6.36	1.11	-179	100	2.3	1,000	6.3	1,190	NA	6,900	0.008	0.180
	11-Nov-03	6.43	0.57	-165	68	3.2	1,200	3.6	2,040	NA	4,000	0.054	0.190
(USEPA)	14-Jul-04	6.35	0.86	-163	118	2.7	1,240	0.25	2,240	NA	5,560	<2.0	<2.7
	20-Apr-05	6.37	0.4	-159	57	1.7	1,600	0.03	1,360	NA	13,000	<0.005	0.180
MP09	17-Jul-02	7.03	0.21	-6	17	1.3	1,800	1.6	220	NA	47	0.069	0.290
	19-Sep-02	7.01	<0.01	-161	25	0.9	1,100	<0.01	400	NA	3,300	0.065	0.530
	19-Mar-03	6.55	1.34	-8	43	0.14	860	<0.01	952	NA	7,600	0.015	0.120
	10-Nov-03	6.39	0.57	-90	14	0.66	1,300	0.20	1,020	NA	3,000	0.094	0.140
(USEPA)	14-Jul-04	6.46	0.45	-135	8.7	1.6	1,390	<0.10	1,010	NA	1,370	<2.0	<2.7
	20-Apr-05	6.39	0.40	-22	26	NA	1,100	NA	NA	NA	1,800	<0.005	0.087
(USEPA)	11-Apr-07	6.56	1.57	-46	3.83	0.15	1,700	<0.01	620	NA	69	<1.9	<2.6
MP10	19-Jul-02	7.15	8.3[g/]	45	<5.0	<0.01	2,200	0.90	120	NA	5.2	0.110	0.870
	19-Sep-02	7.22	4.18	72	<5.0	0.03	2,100	0.60	220	NA	0.99	0.068	0.340
	19-Mar-03	6.93	3.37	70	<5.0	0.08	2,500	<0.01	204	NA	24	0.120	0.071
	10-Nov-03	6.77	2.56	70	2.9J	0.19	2,600	0.20	238	NA	9.2	0.079	0.033
(USEPA)	14-Jul-04	6.82	0.51	-121	7.2	<0.2	2,080	<0.10	320	NA	30	<2.0	<2.7
	20-Apr-05	6.71	1.00	61	10	<0.2	3,200	<0.10	292	NA	12	0.010	0.014

[a/] su = standard pH units.
[b/] mg/L = milligrams per liter.
[c/] mV = millivolts.
[d/] nM = nanomolar.
[e/] µg/L = micrograms per liter.
[f/] NA = not analyzed.
[g/] Dissolved oxygen data collected at this location may not be representative because the well purged dry.

Ferrous Iron. Concentrations of ferrous iron upgradient of the biowall are less than 0.2 mg/L, and have typically been non-detect. Within the biowall, concentrations of ferrous iron have been slightly elevated, with a maximum concentration of 6.0 mg/L measured at location MP01 in November 2003. Iron may adsorb or precipitate with sulfide to form iron-sulfide minerals. Therefore, concentrations of ferrous iron measured in groundwater may not be an accurate indication of the level of iron reduction that is occurring within the biowall.

Sulfate. Background sulfate levels range from 1,250 mg/L to 2,500 mg/L at locations OU-1-01 and WL019. Sulfate concentrations within the biowall at location MP01 have been depleted to as low as 9.5 mg/L in July 2004. However, sulfate concentrations quickly return to near background concentrations within 5 to 10 feet downgradient of the biowall (locations MP02, MP03, MP07, and MP08). The concentration of sulfate at location MP02 in July 2004 was 1,560 mg/L, compared to 9.5 mg/L in MP01. With an exception for location MP06 in April 2005, concentrations of sulfate have been consistently reduced to less than 500 mg/L at MP01 and to less than 1,000 mg/L at MP06 over the 58 months of post-installation monitoring.

Methane. Background concentrations of dissolved methane have been consistently measured at less that 0.1 mg/L. Concentrations of methane measured in the biowall in April 2005 remain elevated at concentrations of 13 mg/L at MP01 and 14 mg/L at MP06. Concentrations of methane within the biowall in April 2007 decreased to approximately 0.8 mg/L at MP01 and 3.0 mg/L at MP06, and there appears to be a decrease in the level of methanogenic activity within the biowall at 58 months post installation.

Dissolved Hydrogen. Concentrations of dissolved hydrogen measured within and immediately downgradient of the biowall in September 2002, March 2003, and November 2003 ranged from 0.60 to 2.4 nanomoles per liter (nmol/L). Taken in the context of depleted sulfate levels, these concentrations of dissolved hydrogen suggest that sulfate reduction is a predominant redox reaction occurring within the biowall (Lovley *et al*., 1994).

In summary, iron reduction, sulfate reduction and methanogenesis have been induced and sustained within the biowall. Along the northern transect (MP01 to MP05), sulfate levels quickly return to background levels downgradient of the biowall. This trend is less pronounced but still evident along the southern transect (MP06 to MP10). Sulfate levels within the biowall remain depleted in April 2007, indicating the amount of bioavailable substrate has been sufficient to sustain sulfate reduction over a period of 58 months.

4.3 Organic Substrate

The distribution of organic substrate in groundwater may be reflected in levels of TOC (**Table 1**) and metabolic acids (measured as VFAs, data not shown).

Total Organic Carbon. TOC (unfiltered samples) was initially measured within the biowall at concentrations as high as 2,800 mg/L at location MP01 in July 2002, 4 weeks after installation. Over time, concentrations of TOC at location MP01 have steadily decreased to 7.9 mg/L in April 2007. Similarly, concentrations of TOC at location MP06 have steadily decreased from a maximum of 390 mg/L in September 2002 to 14 mg/L in April 2007. Concentrations of TOC greater than 20 to 30 mg/L appear sufficient to sustain methanogenic conditions. As TOC decreased to less than 10 to 20 mg/L in April 2007, the degree of methanogenesis also appears to have decreased. However, sulfate reduction has been sustained.

Background concentrations of TOC in the upgradient monitoring wells are typically less than 10 mg/L. Levels of TOC observed at a distance of 30 feet downgradient from the biowall in April 2005 were 28 mg/L at well MP04 and 26 mg/L at well MP09. This suggests that the anaerobic treatment zone may extend as far as 30 feet downgradient of the biowall. It is interesting to note that in both July 2004 and April 2005 the concentrations of TOC in the wells immediately downgradient of the biowall (*i.e.*, locations MP02, MP07, and MP08) are greater than within the biowall. This may be due to the possibility of a well within the biowall trench collecting groundwater from the upgradient edge of the trench or from the upgradient portion of the aquifer during purging. A well downgradient of the biowall is less likely to produce water that has not been completely exposed to the mulch in the biowall.

Metabolic Acids. Metabolic acids (data not shown) measured in July 2002 were primarily acetic, propionic, and butyric acids. Concentrations of total metabolic acids were 959 mg/L at MP01 and 1,367 mg/L at MP06 in July 2002. Concentrations of total metabolic acids decreased to 12 mg/L at MP01 and 11 mg/L at MP06 in March 2003, and measurements of metabolic acids in November 2003 were below method detection limits. Decreasing trends in metabolic acids are similar to those observed for TOC. It is possible that a large proportion of the metabolic acids produced are being rapidly fermented and depleted within the biowall.

In summary, levels of TOC and metabolic acids were highly elevated immediately after installation of the biowall. This may be due to rapid dissolution of the soluble portion of organic matter that was present in the mulch and compost. Levels of TOC have decreased to 8 to 14 mg/L at 58 months after installation. The biowall has sustained strong anaerobic conditions over a period of approximately 5 years, but replenishment with a supplemental carbon source may be beneficial in the near future.

4.4 Degradation of Chlorinated Ethenes

Table 2 summarizes CAHs detected in groundwater during monitoring from July 2002 to April 2007, a period of 58 months following biowall installation. Well installation and the first groundwater sampling was performed approximately 4 weeks after biowall installation. While true "baseline" conditions for the wells located within or downgradient of the biowall trench not obtained, data from upgradient wells can be used to infer natural background conditions.

The primary contaminants detected at the site include TCE and *cis*-DCE. Background concentrations of TCE ranged up to 8,000 µg/L at upgradient location OU-1-01 in September 2002, and concentrations of *cis*-DCE ranged up to 1,800 µg/L at upgradient location OU-1-01 in November 2003. Lesser concentrations (less than 15 µg/L) of tetrachloroethene (PCE), *trans*-1,2-DCE, 1,1-DCE, vinyl chloride (VC), and chloroform have also been detected in upgradient monitoring locations OU-1-01 and WL019.

During the initial sampling event in July 2002, the ratio of TCE to *cis*-DCE ranged from 25:1 to 1.5:1, with the notable exception of biowall location MP01. The ratio of TCE to *cis*-DCE was less than 0.1:1 at location MP01, indicating that degradation of TCE to *cis*-DCE was stimulated within the biowall within 4 weeks of installation. As of the 3 month monitoring event in September 2002, the trend of decreasing TCE was observed at all locations located within 30 feet downgradient of the biowall. Concentrations of TCE at monitoring wells MP05 and MP10, located 100 feet downgradient of the biowall, steadily declined from September 2002 to July 2004 before moderating in April 2005. As of April 2005, concentrations of TCE have been sustained at less than 5.0 µg/L at locations MP01, MP02, MP07, and MP08.

TABLE 2
CHLORINATED ALIPHATIC HYDROCARBONS IN GROUNDWATER

Sample Identification	Sampling Location	Sample Date	Months from Installation	PCE (µg/L)[a/]	TCE (µg/L)	1,1-DCE (µg/L)	*cis*-1,2-DCE (µg/L)	*trans*-1,2-DCE (µg/L)	VC (µg/L)
Northern Flow Path									
OU-1-01	Upgradient	19-Jul-02	1	<9.3	6,200	2.5 J[b/]	850	9.8	<7.3
	of MP01	18-Sep-02	3	<31	8,000	4.1 J	1,100	13	<24
		20-Mar-03	9	<7.0	7,200	3.4 J	1,300	13	0.27 J
		11-Nov-03	17	<14	5,700	4.7 J	1,800	14	<11
(USEPA)		12-Jul-04	25	<1.0	2,590	1.7	811	6.9	0.33 J
(OU1-04)		19-Apr-05	34	<4.6	1,500	<2.2	550	6.4	<3.1
(USEPA)		11-Apr-07	58	--	239	1.2	550	6.1	12
MP01	Within Biowall	18-Jul-02	1	<5.6	48	<4.8	680	1.8 J	<4.4
		18-Sep-02	3	<2.8	0.12 J	<2.4	480	0.76 J	2.5
		20-Mar-03	9	<1.4	0.70 J	<1.2	250	0.44 J	2.0
		12-Nov-03	17	<2.8	<2.0	<2.4	310	0.63 J	2.1 J
(USEPA)		12-Jul-04	25	<1.0	0.74J	<1.0	705	3.1	2.8
		19-Apr-05	34	<1.2	<0.78	<0.55	69	5.5 J	590
(USEPA)		11-Apr-07	58	--	0.38 J	<0.5	36	7.6	113
MP02	5' Downgradient	18-Jul-02	1	0.10 J	290	<1.2	49	0.6	<1.1
	of MP01	18-Sep-02	3	<3.5	55	1.4 J	770	3.6	0.64 J
		20-Mar-03	9	0.087 J	170	1.1 J	610	5.6	0.75 J
		12-Nov-03	17	<5.6	37	2.3 J	1,700	19	3.2 J
(USEPA)		12-Jul-04	25	<1.0	20	0.92 J	876	10	3.0
		19-Apr-05	34	<1.2	<0.78	<0.55	300	11 J	380
MP03	10' Downgradient	18-Jul-02	1	0.18 J	350	<1.2	22	0.39 J	<1.1
	of MP01	18-Sep-02	3	<5.6	150	3.7 J	1,200	8.5	0.27 J
		20-Mar-03	9	<2.8	160	1.3 J	510 M	4.4	0.38 J
		12-Nov-03	17	<3.5	100	2.2 J	1,200	21	1.9 J
(USEPA)		12-Jul-04	25	<1.0	37	0.81 J	972	8.0	3.8
		19-Apr-05	34	<2.3	10.1 J	<1.1	2,095	31	259
MP04	30' Downgradient	18-Jul-02	1	0.20 J	430	<1.2	260	2.1	0.055 J
	of MP01	18-Sep-02	3	<5.6	120	3.0 J	1,100	8.8	0.35 J
		20-Mar-03	9	<7.0	130	2.4 J	1,200	15	1.2 J
		11-Nov-03	17	<14	120	4.8 J	3,400	41	4.3 J
(USEPA)		12-Jul-04	25	<0.19	89	3.4	2,800	63	3.8
		19-Apr-05	34	<2.3	174	<1.1	1,170	71	120
(USEPA)		11-Apr-07	58	--	79	0.80	308	46	236
MP05	100' Downgradient	18-Jul-02	1	0.37 J	2,500	1.1 J	240	15	<5.5
	of MP01	19-Sep-02	3	<16	3,000	2.5 J	590	25	<12
		19-Mar-03	9	0.28 J	2,000	3.7	1,500	31	0.60 J
		10-Nov-03	17	<7.0	1,600	4.9 J	1,600	42	1.3 J
(USEPA)		12-Jul-04	25	<1.0	569	5.2	2,660	54	1.7
		20-Apr-05	34	<4.6	581	<2.2	2,461	77 J	<3.1

(continued)

TABLE 2 (concluded)
CHLORINATED ALIPHATIC HYDROCARBONS IN GROUNDWATER

Sample Identification	Sampling Location	Sample Date	Months from Installation	PCE (µg/L)[a/]	TCE (µg/L)	1,1-DCE (µg/L)	*cis*-1,2-DCE (µg/L)	*trans*-1,2-DCE (µg/L)	VC (µg/L)
Southern Flow Path									
WL019	Upgradient	19-Jul-02	1	0.28 J	1,500	0.74 J	130	3.0	<4.4
	of MP06	19-Sep-02	3	0.19 J	1,200	0.82 J	140	7.6	<3.7
		19-Mar-03	9	0.23 J	1,300	0.60 J	130	2.6	0.076 J
		11-Nov-03	17	0.18 J	1,300	0.67 J	150	4.1	<2.2
(USEPA)		12-Jul-04	25	<0.19	812	0.38 J	79	3.3	<0.30
		18-Apr-05	34	<0.12	74	0.46 J	73	16	<0.78
MP06	Within Biowall	18-Jul-02	1	<2.8	170	<2.4	80	2.8	<2.2
		17-Sep-02	3	<2.8	5.2	<2.4	310	2.7	1.5 J
		18-Mar-03	9	<1.4	2.0	0.12 J	360	7.6	<1.1
		11-Nov-03	17	<1.4	0.44 J	<1.2	290	13	3.1
(USEPA)		12-Jul-04	25	<1.0	3.1	<0.28	164	23	4.0
		18-Apr-05	34	<0.23	5.8	<0.11	85	24	3.0 J
(USEPA)		11-Apr-07	58	--	2.1	<0.50	68	32	41
MP07	5' Downgradient	19-Jul-02	1	0.051 J	190	<1.2	130	6.3	<1.1
	of MP06	17-Sep-02	3	<2.8	10	<2.4	300	4.6	0.88 J
		18-Mar-03	9	<1.4	2.6	0.42 J	290	16	2.6
		11-Nov-03	17	<1.4	1.7	0.47 J	370	22	3.8
(USEPA)		12-Jul-04	25	<1.0	2.3	0.34 J	266	20	3.2
		18-Apr-05	34	<0.46	<0.31	<0.22	190	28	4.6 J
MP08	10' Downgradient	19-Jul-02	1	0.063 J	250	<1.2	130	7.0	0.16 J
	of MP06	19-Sep-02	3	<1.4	4.5	0.58 J	330	5.5	0.90 J
		18-Mar-03	9	<1.4	4.6	0.55 J	320	14	2.2
		11-Nov-03	17	<2.8	1.2 J	0.61 J	540	22	4.1
(USEPA)		12-Jul-04	25	<1.0	4.7	0.40 J	367	19	4.2
		20-Apr-05	34	<0.46	3.32 J	<0.22	272	32	5.39 J
MP09	30' Downgradient	17-Jul-02	1	<1.4	220	0.56 J	150	9.9	0.19 J
	of MP06	19-Sep-02	3	<1.4	69	0.69 J	200	17	0.38 J
		19-Mar-03	9	<1.4	17	0.49 J	200	24	1.6
		10-Nov-03	17	<1.4	6.3	0.55 J	290	35	2.4
(USEPA)		12-Jul-04	25	<1.0	2.3	0.42 J	188	43	1.6
		20-Apr-05	34	<0.92	7.67 J	<0.44	314	80	3.7 J
(USEPA)		11-Apr-07	58	--	7.5	0.78	217	94	2.6
MP10	100' Downgradient	19-Jul-02	1	0.47 J	670	0.49 J	27	2.4	<2.2
	of MP06	19-Sep-02	3	0.28 J	460	0.50 J	32	4.7	<2.2
		19-Mar-03	9	0.28 J	390	0.53 J	42	21	0.13 J
		10-Nov-03	17	0.13 J	330	0.29 J	48	20	<1.1
(USEPA)		12-Jul-04	25	<1.0	236	0.31 J	63	44	<0.30
		20-Apr-05	34	<0.92	407	<0.44	79	26	<0.62

[a/] µg/L = micrograms per liter.

[b/] J-flag indicates the concentration is estimated.

From July 2002 to November 2003, concentrations of *cis*-DCE generally increased across the biowall monitoring network, both upgradient and downgradient of the biowall. From November 2003 to April 2005 the concentrations of *cis*-DCE in the upgradient wells generally decreased, but were variable across the remainder of the monitoring network. Within the biowall at location MP01, the concentration of *cis*-DCE peaked at 705 µg/L in July 2004, before decreasing to 69 µg/L in April 2005 and 36 µg/L in April 2007. For biowall location MP06, the concentration of *cis*-DCE peaked at 360 µg/L in March 2003, and then decreased to 85 µg/L in April 2005 and 68 µg/L in April 2007. Note that concentrations of *cis*-DCE also decreased in upgradient wells OU-1-01 and WL019 after November 2003.

VC had not accumulated prior to April 2005, when VC was observed at a concentration of 590 µg/L at location MP01. Some VC may be attributed to the production of VC in the upgradient bioreactor where a much longer residence time in the reaction zone is achieved due to groundwater recirculation (**Appendix F.3**). However the concentrations of VC within and downgradient of the biowall are clearly elevated relative to upgradient locations for the northern transect in April 2005, and at both transects in April 2007. This suggests that the microbial consortia in the biowall has adapted to the biotic dechlorination of DCE to VC, although further dechlorination to ethene not evident (**Table 1**). Therefore, the degree of biotic dechlorination of DCE to VC appears to be increasing relative to biogeochemical transformation processes that do not produce VC. It is notable that elevated concentrations of VC have not been observed at downgradient locations MP05 and MP10, suggesting that VC is attenuated within a short distance (100 feet) of the biowall.

Table 3 lists the concentrations of TCE and total molar concentrations of chloroethenes (PCE, TCE, DCE, and VC) through April 2005 for upgradient wells OU-1-01 and WL019 and monitoring locations MP01 and MP06 located within the biowall trench. Percent reductions were calculated for concentrations in the biowall relative to the upgradient monitoring locations. The average decrease in the concentration of TCE in April 2005 was 96 percent. There appears to be little decrease in the effectiveness of the biowall to degrade TCE over time. Concentrations of TCE within the biowall continued to be less than 5.0 µg/L in April 2007, 58 months after installation.

Percent reductions in total molar concentrations of chloroethenes over time along the northern and southern transects range from approximately 18 to 96 percent. The average reduction in total molar concentration of chloroethenes within the biowall was 73 percent in July 2004, but decreased to approximately 31 percent in April 2005. It should be noted that the transformation of TCE to *cis*-DCE or of *cis*-DCE to VC alone would not result in a reduction in the total molar concentration of chloroethenes. Therefore, the reduction in total molar concentration of chloroethenes through July 2004 (predominately TCE and *cis*-DCE) indicates that *cis*-DCE is also being degraded, with production of only low levels of VC or ethene. As the production of VC increased in the northern transect in April 2005, the reduction in total molar concentration also decreased. This suggests an increase in the extent of biotic reductive dechlorination of *cis*-DCE to VC, relative to biogeochemical transformation.

Figure 4 and **Figure 5** show total molar concentrations along the northern and southern well transects for each sampling event through April 2005. Total molar concentrations would be expected to remain constant if TCE was simply being transformed to *cis*-DCE without any additional degradation of *cis*-DCE. However, total molar concentrations of chloroethenes are clearly depleted within the biowall.

TABLE 3
PERCENT REDUCTIONS IN TCE AND TOTAL CHLOROETHENES

Reductions in Concentration of TCE[a/]

	Northern Flow Path			Southern Flow Path			Average of Flow Paths
Date	TCE OU-1-01 (µg/L)[b/]	TCE PES-MP01 (µg/L)	Percent Reduction TCE	TCE WL-019 (µg/L)	TCE PES-MP06 (µg/L)	Percent Reduction TCE	Percent Reduction TCE
19-Jul-02	6,200	48	**99.2**	1,500	170	**88.7**	**93.9**
18-Sep-02	8,000	0.12	**99.999**	1,200	5.2	**99.6**	**99.8**
20-Mar-03	7,200	0.70	**99.99**	1,300	2.0	**99.8**	**99.9**
11-Nov-03	5,700	<2.0	**99.98**	1,300	0.44	**99.97**	**99.97**
12-Jul-04	2,590	0.74	**99.97**	812	3.1	**99.6**	**99.8**
19-Apr-05	1,500	<0.78	**99.97**	74	5.8	**92.2**	**96.1**

Reductions in Molar Concentration of Total Chloroethenes

	Northern Flow Path			Southern Flow Path			Average of Flow Paths
Date	Total Molar Chlorethenes OU-1-01 (nmol/L)[c/]	Total Molar Chlorethenes PES-MP01 (nmol/L)	Percent Reduction Total Molar Chloroethenes	Total Molar Chlorethenes WL-019 (nmol/L)	Total Molar Chlorethenes PES-MP06 (nmol/L)	Percent Reduction Total Molar Chloroethenes	Percent Reduction Total Molar Chloroethenes
19-Jul-02	56,144	7,432	**86.8**	12,825	2,174	**83.0**	**84.9**
18-Sep-02	72,697	5,017	**93.1**	10,695	3,298	**69.2**	**81.1**
20-Mar-03	68,404	2,625	**96.2**	11,271	3,833	**66.0**	**81.1**
11-Nov-03	62,273	3,254	**94.8**	11,509	3,183	**72.3**	**83.6**
12-Jul-04	28,175	7,358	**73.9**	7,036	2,020	**71.3**	**72.6**
19-Apr-05	17,789	10,215	**42.6**	1,492	1,217	**18.4**	**30.5**

[a/] TCE = trichloroethene

[b/] µg/L = micrograms per liter.

[c/] nmol/L = nanomoles per liter.

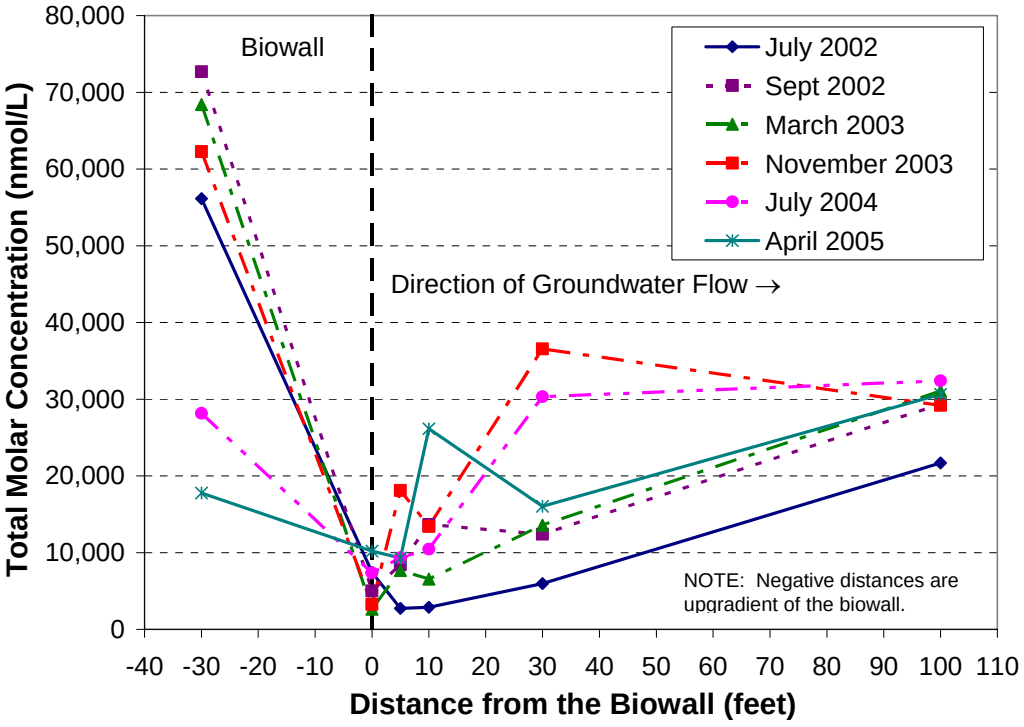

Figure 4. Total Molar Concentration of Chloroethenes along the Northern Transect

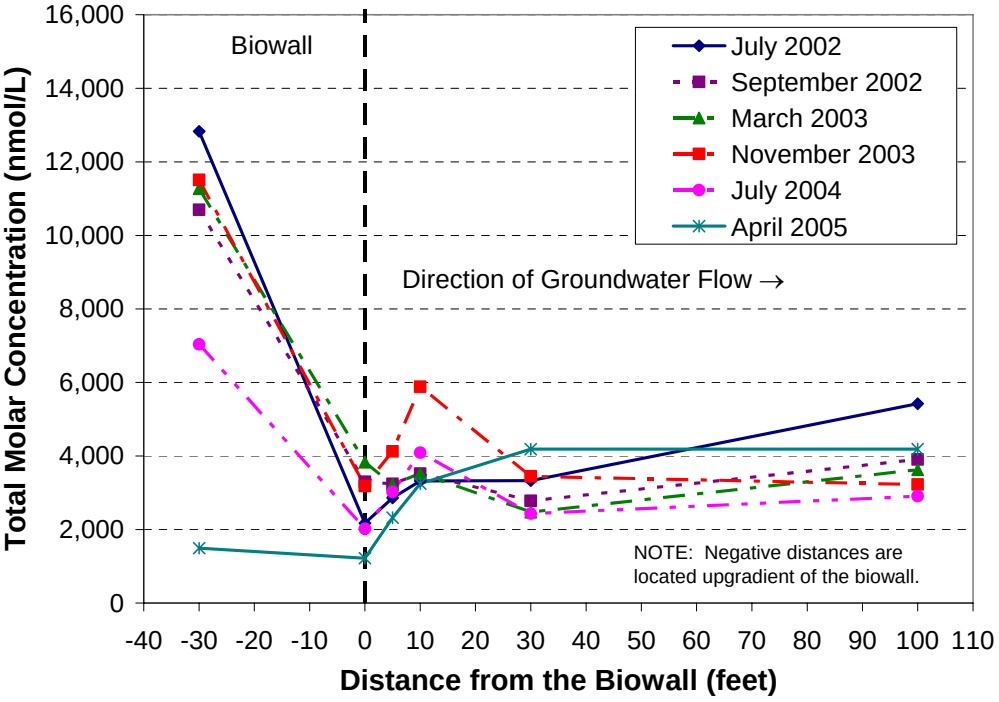

Figure 5. Total Molar Concentration of Chloroethenes along the Southern Transect

Total molar concentrations upgradient of the biowall show a reduction of 50 percent or more in July 2004 and April 2005. This may be due, in part, to reductions in contaminant concentrations achieved in the LF-03 source area bioreactor (**Appendix F.3**). The biowall should continue to be effective in degrading TCE and *cis*-DCE as long as favorable geochemical conditions can be maintained.

An increase in total molar concentration downgradient of the biowall may be due to the continued desorption of CAHs from downgradient soils, mixing with untreated groundwater, or indicate that degradation may be limited to the immediate biowall reactive zone. It is possible that at least a portion of the increase in concentrations of *cis*-DCE downgradient of the biowall are due to desorption of TCE and transformation to *cis*-DCE. While the transformation of TCE to DCE may result in an apparent accumulation of *cis*-DCE in some locations, there remains a significant overall loss of chloroethene mass over time within the biowall.

While the transformation of TCE to *cis*-DCE could be attributed to biological reductive dechlorination, the reduction in total molar concentrations and a general lack of VC and ethene suggests that the predominant degradation process is biogeochemical reduction in the presence of reactive metal-sulfide minerals produced under anaerobic conditions (Kennedy and Everett, 2003; Shen and Wilson, 2007). The USEPA NRMRL/GWERD in Ada, Oklahoma is currently investigating degradation processes at the LF-03 biowall and is collaborating with the Air Force to more fully describe the fate of CAHs in the OU-1 aquifer system.

Figure 6 shows molar concentrations of TCE, total DCE, VC and ethene plus ethane over distance along the northern transect for data collected in November 2003. Both TCE and *cis*-DCE are greatly reduced within the biowall. Downgradient of the biowall the total molar concentration is comprised almost entirely of *cis*-DCE for a distance of 30 feet. The relative concentration of chloroethenes within the biowall is consistent with biogeochemical reduction, where intermediate dechlorination products do not accumulate. The degradation signature downgradient of the biowall is more consistent with sequential dechlorination of TCE to *cis*-DCE, with accumulation of *cis*-DCE. Further dechlorination to VC or ethene is not evident.

For the southern transect (**Figure 7**), TCE is greatly reduced within the biowall but the concentration of *cis*-DCE increases relative to the upgradient concentration. The total molar concentration is still greatly reduced in the biowall. In this case both biogeochemical reduction and sequential transformation of TCE to *cis*-DCE may be occurring.

The occurrence of biogeochemical reduction is supported by the presence of high concentrations of sulfides measured in samples collected from the biowall. **Table 4** lists concentrations of iron and sulfides measured in samples of biowall material collected from two soil borings in April 2005. Soil boring number 1 (SB1) was drilled adjacent to monitoring location MP01, and soil boring SB2 was drilled adjacent to location MP06.

For boring SB-1, the concentration of bioavailable ferric iron ranged from 441 to 645 milligrams per kilogram (mg/kg). A weak acid extraction was also performed as an approximation of bioavailable ferric iron and biogenic ferrous iron. Weak acid extractable ferric iron was less than 200 mg/kg, indicating that the weak acid extraction method may not be suitable to estimate the amount of bioavailable ferric iron in the biowall materials. However, weak acid extractable ferrous iron was measured as high as 1,900 mg/kg. This suggests that the majority of bioavailable ferric iron has been reduced to biogenic ferrous iron.

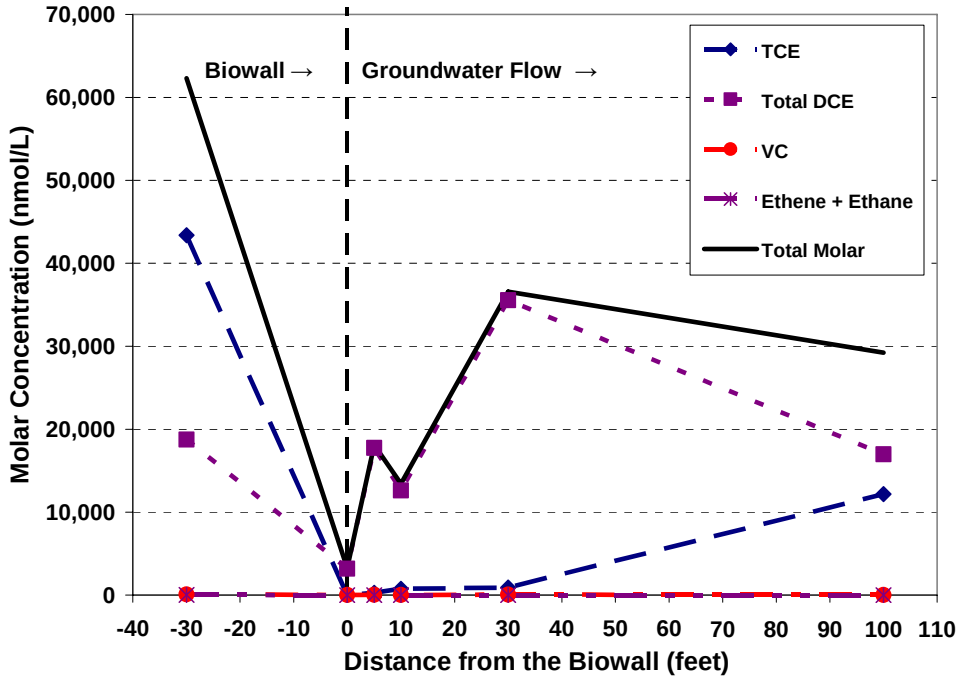

Figure 6. Concentrations of Chloroethenes along the Northern Transect in November 2003

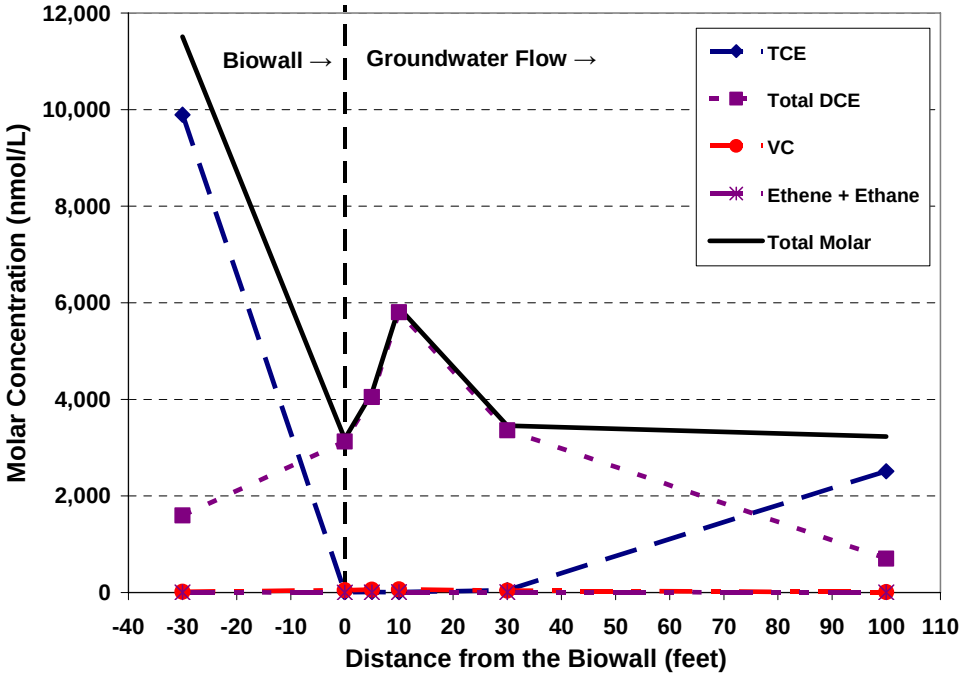

Figure 7. Concentrations of Chloroethenes along the Southern Transect in November 2003

TABLE 4

SUMMARY OF SOIL/MULCH BIOGEOCHEMICAL ANALYTICAL RESULTS

Sample Location	Sample Date	Sample Depth (feet bgs)[a/]	Percent Solids by Weight	Organic Carbon (mg/kg)[b/]	Bioavailable Fe^{3+} (mg/kg)	$WAEFe^{3+}$ [c/] (mg/kg)	$WAEFe^{2+}$ [c/] (mg/kg)	$SAEFe^{3+}$ [c/] (mg/kg)	$SAEFe^{2+}$ [c/] (mg/kg)	AVS [c/] (mg/kg)	CES [b/] (mg/kg)
SB1-5	22-Apr-05	5	60%	29,000	441	<200[d/]	1,300	<300	3,700	13,000	19,000
SB1-15	22-Apr-05	15	63%	41,000	622	<200	1,900	<300	5,200	13,000	9,800
SB1-20	22-Apr-05	20	64%	21,000	645	<200	<200	<300	500	6,900	7,800
SB2-7	22-Apr-05	7	86%	15,000	290	<100	300	<200	1,000	9,000	6,400
SB12-7 (dup)	22-Apr-05	7	67%	23,000	14	<100	600	400	1,800	14,000	12,000
SB2-15	22-Apr-05	15	78%	18,000	182	<100	1,200	<200	3,100	9,400	2,400
SB2-20	22-Apr-05	20	78%	20,000	<6.4	<100	3,100	900	5,400	7,400	8,200

Note: Soil samples analyzed by Microseeps, Inc. of Pittsburgh, Pennsylvania.

[a/] feet bgs = feet below ground surface.

[b/] mg/kg = milligrams per kilogram dry weight.

[c/] $WAEFe^{3+}$ = weak acid extractable ferric iron; $SAEFe^{3+}$ = strong acid extractable ferric iron; $WAEFe^{2+}$ = weak acid extractable ferrous iron; $SAEFe^{2+}$ = strong acid extractable ferrous iron; AVS = acid volatile sulfide; CES = chromium extractable sulfide.

[d/] <200 indicates that the analyte was not detected above the indicated method detection limit.

A strong acid extraction was performed as an approximation of the total amount of ferric and ferrous iron in the biowall samples. Strong acid extractable ferric iron was less than 300 mg/kg in SB-1, while strong acid extractable ferrous iron ranged from 500 to 3,700 mg/kg. This also suggests that most bioavailable ferric iron has been reduced to biogenic ferrous iron. The source of iron in the biowall is likely iron oxide coatings on the river sand used for biowall construction.

Acid volatile sulfide (AVS) extraction is used as an approximation of reduced sulfide in the form of a metal mono-sulfide, or in this case an approximation of iron mono-sulfide (FeS). Concentrations of AVS in SB1 ranged from 6,900 to 13,000 mg/kg. Chromium extractable sulfide (CES) extraction is performed to measure the total amount of sulfides in a sample. When performed following AVS extraction, it is an approximation of the amount of elemental sulfur and metal disulfide (FeS_2) in the sample. CES ranged from 7,800 to 19,000 in the samples from SB1.

Results for SB2 were similar, although the magnitude of the concentrations of bioavailable ferric iron were lower. Concentrations of AVS ranged from 7,400 to 14,000 mg/kg, similar in magnitude to SB1. For comparison, the maximum concentration of AVS measured by Kennedy and Everett (2003) for samples of the biowall material was 674 mg/kg. Kennedy and Everett (2003) measured a concentration of AVS of 0.40 mg/kg for biowall fill material above the water table, assumed to be representative of the chemical state of the backfill material when the biowall was constructed. For both sample sets, the amount of AVS is highly elevated within the biowall due to the processes of iron and sulfate reduction.

Concentrations of FeS estimated from measurements of AVS appear to be sufficient to degrade the flux of TCE migrating through the biowall based on stoichiometric relationships (**Appendix D**). However, these relationships do not account for the rate at which the reaction of TCE with FeS may occur. Shen and Wilson (2007) extracted rates of abiotic degradation of TCE with FeS of 0.53 to 2.3 per day per mole of FeS in contact with 1.0 liter of pore water. Using laboratory measured rates of the degradation of TCE per mole of FeS in contact with 1.0 liter of pore water is one method to estimate the contribution of FeS to the sustained degradation of TCE in the biowall.

The concentrations of FeS presented in **Table 4** are equivalent to near 1.0 mole FeS in contact with 1.0 liter pore water (see calculations in **Appendix D**). Given an estimate of groundwater residence time of 15 days, and the slower rate constant (0.53 per day) from Shen and Wilson (2007), a first order law would predict a concentration exiting the biowall of less than 0.0004 of the influent concentration. Comparing concentrations of TCE at well locations 10 feet down gradient of the biowall to wells upgradient of the biowall in July 2004 and April 2005 indicates that the downgradient concentration of TCE is approximately 1 percent of the upgradient concentration (a 99 percent reduction). This reduction may be explained by the reaction of TCE with FeS using rates observed in the laboratory column studies.

5. CAPITAL CONSTRUCTION AND MONITORING COSTS

Approximate costs to install and monitor the biowall include $10,000 for design and work plan development; $169,000 for procurement, mobilization, trench installation, and monitoring well installation; $57,000 for the first three rounds of process monitoring; and $34,000 for reporting and meetings. The trenching subcontract was approximately $115,000. Total cost for

design, installation, one year of semi-annual monitoring and reporting is approximately $270,000. Capital cost ($169,000) for installation of the biowall is approximately $370 per linear foot, including all materials, labor, and installation of the monitoring network. Future operations and maintenance (O&M) costs are anticipated to be approximately $25,000 per year for annual monitoring and reporting. Recharge of the biowall may be required to maintain performance, and is not included in the cost of O&M.

6. SUMMARY

Geochemical data indicate that levels of organic carbon within the biowall are sufficient to induce and sustain sulfate reduction and methanogenesis over a period of 58 months. These oxidation-reduction conditions are conducive to anaerobic degradation of CAHs. TCE has been reduced to below the USEPA drinking water maximum contaminant level (MCL) of 5.0 µg/L within the biowall, with the exception of 5.8 µg/L at location MP06 in April 2005.

Relative to reductions in concentrations of TCE, *cis*-DCE did not accumulate in the biowall and only low concentrations of VC and ethene were observed prior to April 2005. This suggests that biogeochemical transformation within the biowall trench is the primary degradation process due to reaction of TCE and *cis*-DCE with reduced metal-sulfides (Kennedy and Everett, 2003). Concentrations of AVS measured in biowall samples are sufficient to account for the observed reductions in concentrations of TCE.

Downgradient of the biowall in the northern transect, the concentration *cis*-DCE was observed to increase by an order of magnitude or more at all locations relative to concentrations measured in July 2002. This suggests that biotic reductive dechlorination of TCE to *cis*-DCE is likely a predominant degradation pathway downgradient of the biowall, but that the process is incomplete and *cis*-DCE is not further reduced to VC and ethene. The increase in concentrations of *cis*-DCE downgradient of the biowall in the southern transect is less pronounced.

VC had not accumulated prior to April 2005, when VC was observed at a concentration of 590 µg/L at location MP01. While some VC may be attributed to an upgradient bioreactor, concentrations of VC within and downgradient of the biowall are clearly elevated relative to upgradient locations for the northern transect in April 2005, and for both transects in April 2007. This suggests that the microbial consortia in the biowall has adapted to the biotic dechlorination of DCE to VC, although further dechlorination to ethene not evident. Therefore, the degree of biotic dechlorination of DCE to VC appears to be increasing relative to biogeochemical transformation processes that do not produce VC. Elevated concentrations of VC have not been observed at downgradient locations MP05 and MP10, suggesting that VC is attenuated within a short distance of the biowall.

Evaluation of CAH concentrations along a transect or over time are complicated by 1) changes in concentrations of TCE, *cis*-DCE and VC upgradient of the northern transect due to installation of a bioreactor, 2) steadily decreasing concentrations of TCE and *cis*-DCE upgradient of the southern transect, and 3) aquifer heterogeneity, preferential flow paths, and seasonable variations in groundwater flow. Nonetheless, an average decrease of over 96 percent in the concentration of TCE was observed within the biowall over the first 58 months of monitoring following installation of the biowall.

Additional monitoring is required to document the ability of the biowall to sustain biological activity and degradation of TCE and *cis*-DCE over time. There is little data in the literature to

estimate what the longevity or long-term efficiency of the mulch biowall may be. Other investigators have installed bioreactors filled with a variety of waste cellulose solids (*e.g.*, sawdust and mulch) for the treatment of nitrate-contaminated water and have found little reduction in performance during 7 years of operation (Robertson *et al*., 2000). Based on data at 58 months post installation, it appears the OU-1 biowall may need to be recharged every 5 to 6 years. The life of a biowall system can be extended by periodically recharging the wall with a fluid substrate such as emulsified vegetable oil. This recharge option is relatively inexpensive compared to construction cost, and is simple to complete as an infrequent biowall maintenance event.

5. ACKNOWLEDGEMENTS

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APPENDIX F.3

DEMONSTRATION OF A RECIRCULATION BIOREACTOR AT LANDFILL 3, ALTUS AIR FORCE BASE, OKLAHOMA

DEMONSTRATION OF A RECIRCULATION BIOREACTOR AT LANDFILL 3, ALTUS AIR FORCE BASE, OKLAHOMA

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A pilot-scale recirculation bioreactor was constructed in October 2003 at Altus Air Force Base (AFB), Oklahoma, Landfill 3 (LF-03). The purpose of constructing and operating the bioreactor was to demonstrate the degree to which a combination of organic material (mulch) and accelerated leaching of soluble organic carbon can reduce source area groundwater concentrations of chlorinated aliphatic hydrocarbons (CAHs) in unlined, closed landfills (or other CAH-contaminated sites).

Five initial performance monitoring events were conducted over a period of approximately 24 months from November 2003 to November 2005 (Parsons, 2006). Bioreactor removal efficiencies for trichloroethene (TCE) and total chlorinated ethenes (sum of TCE, dichloroethene isomers [DCE], and vinyl chloride [VC]) from recirculated groundwater ranged from 97 to 100 percent and from 76 to 96 percent, respectively. Over the initial 2-year period of operation at least 6.5 pounds of TCE was removed from the influent water to the bioreactor.

A bioenhancement of the bioreactor was performed in October 2006, following a groundwater sampling event and maintanance (cleaning) of the recirculation system in July 2006 (Parsons, 2008). Bioenehancement consisted of injecting emulsified vegetable oil and a commercial bioaugmentation culture through the recirculation drip lines. Additional performance monitoring was conducted at 3 months (January 2007) and 9 months (July 2007) following the bioenhancement injection. The bioreactor continues to operate with minimal maintenance of the solar powered recirculation system.

Recirculating bioreactors are expected to be a cost-effective full-scale remediation technology, and a full-scale bioreactor has been installed at the Spill Site 17 (SS-17) source area at Altus AFB. Costs associated with full-scale recirculation bioreactor installation and operation are estimated to be similar to source area treatment with organic substrate injection, and considerably less than a Resource Conservation and Recovery Act (RCRA) cap with associated leachate collection and treatment.

1.0 SITE DESCRIPTION

Altus AFB is located in southwestern Oklahoma, approximately 130 miles from Oklahoma City. The base occupies an area of over 2,500 acres and is bordered by the city of Altus on the west, Highway 62 on the south, and agricultural land on the north and east. The base is located approximately 1,300 to 1,400 feet above mean sea level in the Central Redbed Plains region, characterized by a gently sloping land surface. The climate in the region is semi-arid, with cold winters and long hot summers. Precipitation at the base averages approximately 25 inches per year, primarily occurring during spring thunderstorms. Annual potential evaporation usually exceeds precipitation. LF-03 is located within Operable Unit 1 (OU-1) in the northeastern portion of the Base in a remote area adjacent to airfield taxiways. Vegetation at OU-1 primarily consists of prairie grasses.

From 1956 through 1965, LF-03 received waste materials including garbage, wood, metal, paper, and shop wastes. After 1965, LF-03 received construction debris, concrete, brush, and several drums of paint waste. From 1956 to 1965, waste at LF-03 was buried in trenches with depths ranging from 6 to 8 feet below ground surface (bgs).

1.1 Geology and Hydrogeology

The shallow geology in the area of the bioreactor consists of approximately 10 to 30 feet of fractured red-brown calcareous clay and silty clay. Beneath the alluvium is fractured shale bedrock of the Hennessey Group. Both the alluvium and bedrock formation contain abundant ferric iron, giving the sediment its characteristic red color. Layers of white gypsum, which result in high concentrations of sulfate in groundwater, are also present in the sediments of the Hennessy Group (Parsons, 1999).

Two shallow water-bearing zones have been encountered beneath Altus AFB. The depths of the water bearing zones coincide with two distinct lithologic layers, including less consolidated clay extending to a maximum depth of 30 feet bgs, and the underlying layer of well-cemented, better-lithified shale of the Hennessy Group. Preferential flow paths are present in dissolution features and fractured sediments.

Shallow groundwater at the Base occurs under unconfined conditions and generally flows towards the southeast. Shallow groundwater in the LF-03 bioreactor area occurs at depths of 4 to 5 feet bgs during the wet winter and spring months, and from 5 to 9 feet bgs during the dry summer and fall months. The groundwater surface slopes toward the southeast with an average horizontal hydraulic gradient of approximately 0.003 foot per foot (ft/ft) based on water-level measurements recorded in April 1997 and April 1999 (Parsons, 1999).

The hydraulic conductivity at OU-1 ranges from approximately 8 to 20 feet per day (ft/day) in the fractured clay overburden (upper zone). Using this range of hydraulic conductivity values, a measured lateral hydraulic gradient of 0.003 ft/ft, and an estimated effective porosity of 15 percent, the advective groundwater flow velocity in the overburden clay is calculated to range from approximately 0.16 to 0.40 ft/day, or from 58 to 146 feet per year (ft/yr).

1.2 TCE Source Area and Dissolved Phase Plume

From 1984 to 1999, several remedial investigations were completed at and downgradient of LF-03. Groundwater quality data indicate that TCE and *cis*-1,2-DCE are the most prevalent CAHs in OU-1 groundwater in terms of both areal extent and concentration. In November 1999, TCE was detected in groundwater at a concentration of 27,000 micrograms per liter (µg/L) in well WL250 near the suspected LF-03 source area. The TCE dechlorination product *cis*-1,2-DCE also was detected near the source area at concentrations as high as 2,200 µg/L. The TCE plume originates at LF-03 in the vicinity of monitoring well WL250, and extends southeastward approximately 4,000 feet to the Base's eastern boundary. The LF-03 bioreactor is located immediately upgradient of 'hot-spot' well WL250, as this remains the most likely location for residual CAH source material in the landfill.

2.0 TECHNOLOGY DESCRIPTION

The recirculation bioreactor installed at LF-03 is an application of enhanced anaerobic bioremediation, which seeks to exploit anaerobic biodegradation processes to completely

degrade contaminants to innocuous end products (AFCEE *et al*., 2004). The bioreactor provides a source of leachable (soluble) organic material for the CAH-contaminated aquifer, which is utilized by native microorganisms to create a highly reducing anaerobic treatment zone. Leaching of organic carbon and creation of an anaerobic treatment zone is accelerated by the recirculation of groundwater through the bioreactor.

The organic substrate used in the LF-03 bioreactor is a mixture of wood mulch and cotton gin trash. Sand was added to the mixture to improve hydraulic conductivity and reduce compaction in the test cell. Solid carbon substrates, such as mulch and compost, are intended to be relatively long-lasting, slow-release sources of organic carbon, with anticipated life spans of 5 to 10 years (AFCEE *et al*., 2004). Wood mulch is composed of approximately 40 to 50 percent cellulose, which is a natural polymer of glucose molecules, with the chemical formula $(C_6H_{10}O_5)n$ where n ranges from several hundred for wood pulp to over 6,000 for cotton (Senese, 2005). Cotton is the purest form of cellulose. After cellulose, wood is primarily composed of hemicellulose (20 to 30 percent), and lignin (25 to 30 percent), with lignin being the component of plant cell material most recalcitrant to biodegradation (Richard, 1996).

2.1 Bioreactor Components and Construction

The bioreactor at LF-03 consists of three primary components: 1) an excavated cell that contains the organic backfill material and acts as an infiltration gallery for extracted groundwater, 2) a groundwater extraction trench which is installed downgradient of the cell, and 3) a groundwater distribution system which recirculates the extracted groundwater from the trench to the top of the organic mulch in the treatment cell. These components are described in the following paragraphs. **Figure 1** shows a photograph of the completed bioreactor system.

Figure 1. LF-03 Bioreactor Site. Photograph taken from downgradient side of extraction trench with a view to the northwest.

The bioreactor cell is a 30-foot by 30-foot square excavated with a backhoe to a depth of 11 feet bgs (**Figure 2**). The bioreactor cell backfill material consists of approximately 50 percent wood mulch, purchased locally from tree clearing efforts in and around the city of Altus, Oklahoma. The mulch consisted of chipped tree trunks, branches, twigs, and leaves. Class A concrete sand comprised approximately 40 percent of the mixture, with the remaining volume consisting of cotton gin trash purchased from a local farmer's cooperative. The cotton gin trash consisted of a mix of cotton burrs, cotton, and twigs that remained after the preliminary processing. Cotton gin trash is relatively inexpensive and readily available in cotton producing regions where it is used for fertilizer and cattle feed. These three components were combined using a backhoe and front-end loader and used to backfill the cell. The cell was capped with geotextile fabric and a 2-foot layer of native topsoil.

The groundwater collection trench was excavated 18 feet downgradient of the bioreactor cell using a backhoe. The trench is 2 feet wide, 30 feet long, and 18 feet deep in the center. An 18-inch inside diameter (ID), slotted, polyvinyl chloride (PVC) pipe was installed vertically in the middle of the trench to act as the sump. The entire trench was backfilled around the sump to within two feet of the surface with ½-inch plus washed angular gravel. A solar-powered Grundfos 11SQF-2 submersible pump is installed in the sump. The pump discharges to a drip irrigation distribution system at the top of the bioreactor, directly beneath the topsoil cover.

2.2 Groundwater Monitoring Network

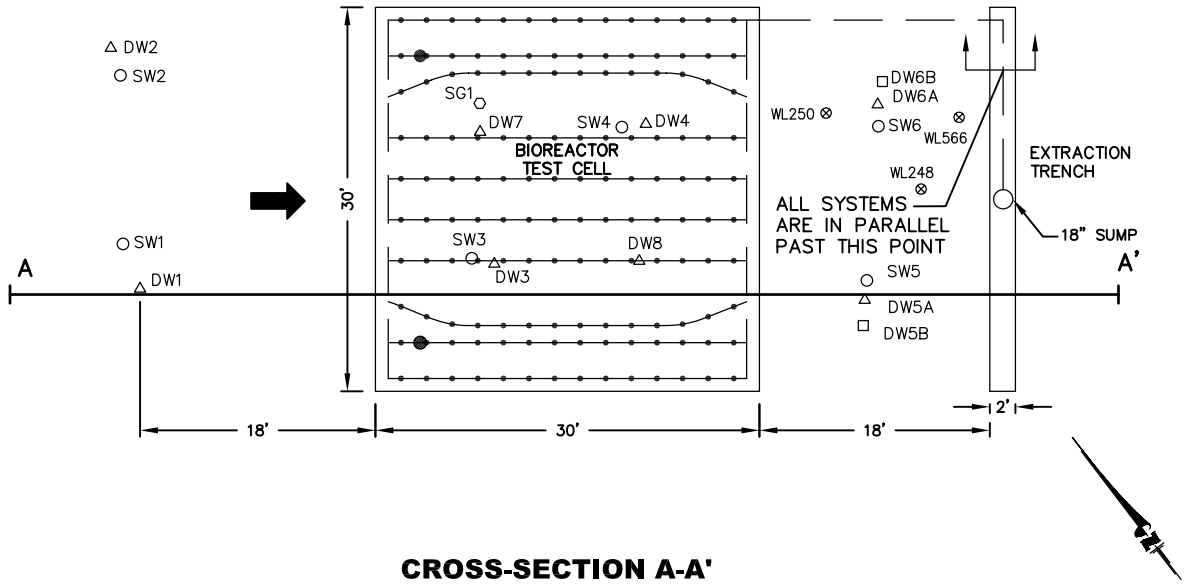
A total of 16, 2-inch ID PVC groundwater monitoring wells are installed within, beneath, and adjacent to the cell. Both shallow (SW) and deep monitoring wells (DW) were installed upgradient, within, and downgradient of the treatment cell. Groundwater samples also were collected from existing downgradient well WL250 and the extraction trench sump. Monitoring wells were installed along two transects oriented parallel to the natural groundwater flow direction. Plan and cross-sectional views of the bioreactor monitoring network are shown on **Figure 2**.

3.0 SYSTEM STARTUP, OPERATION, AND MAINTENANCE

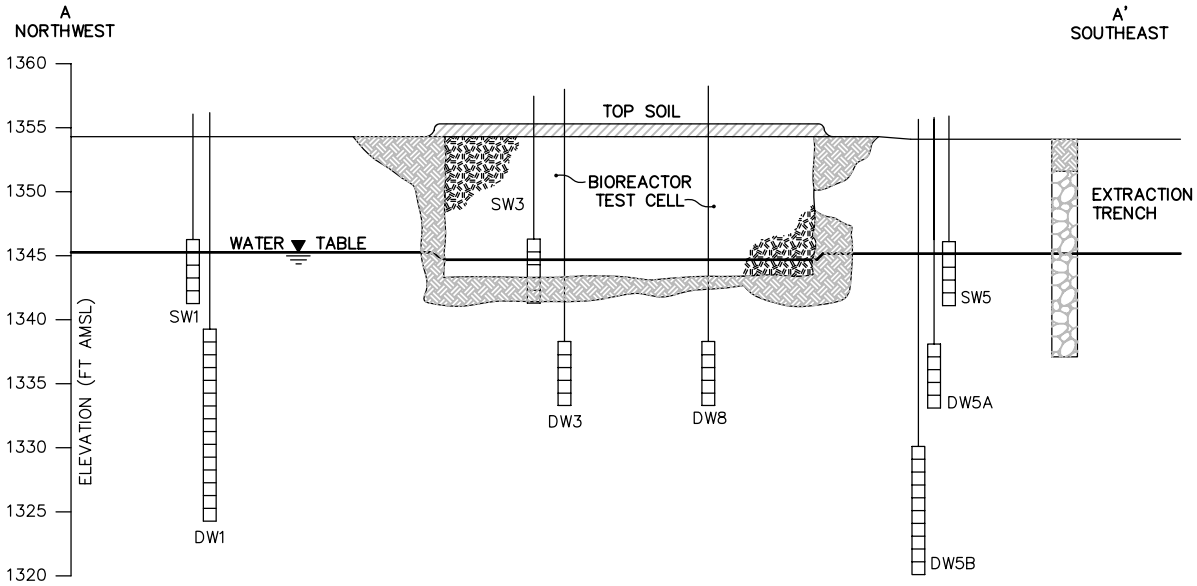
Initial conditions within the bioreactor test cell and the source area aquifer were assessed shortly after bioreactor construction and prior to starting groundwater extraction and recirculation. The baseline groundwater monitoring event was initiated on 06 November 2003, approximately 12 days following completion of test cell construction. System startup occurred after completion of the baseline sampling event, consisting of turning the pump on, checking for leaks, and ensuring that line pressure and flow rate were within the design ranges.

Parsons operated, maintained, and monitored the bioreactor cell at LF-03 from 16 November 2003 through the final demonstration performance monitoring event was completed on 11 November 2005. In addition, two lysimeters installed at the downstream ends of two of the drip irrigation lines were periodically checked to confirm that re-circulated water was being distributed across the bioreactor. During the 2-year demonstration, the pumping rate ranged from 601 to 1,645 gallons per day (gpd) with an overall average of 922 gpd and standard deviation of 201 gpd. Because the pump is solar powered, the pumping rate was directly affected by the intensity of the solar radiation, and groundwater extraction ceased during the night and during times of heavy overcast skies. Maintenance of the system was limited to cleaning the in-line strainer, flushing sediment out of the distribution line, and replacing a faulty valve.

PLAN VIEW



CROSS-SECTION A-A'



LEGEND

- MULCH/SAND MIXTURE
- SW3 ○ SHALLOW MONITORING WELL
- DW3 △ INTERMEDIATE MONITORING WELL
- DW5B □ DEEP MONITORING WELL
- SG1 ⬡ SOIL GAS WELL
- WL524 ⊗ PRE-EXISTING MONITORING WELL (NOT SHOWN ON CROSS-SECTION)
- ● LYSIMETER
- SOLID 17MM POLYETHYLENE TUBING
- 17MM BIOLINE, DRIPPERS @ 24" SPACING, DELIVERS 1 GPH PER DRIPPER
- GROUNDWATER FLOW DIRECTION
- MONITORING WELL WITH SCREEN INTERVAL — DW7

0' 15'

SCALE: HORIZONTAL 1"=15'
VERTICAL 1"=15'

FIGURE 2

PLAN VIEW AND CROSS-SECTION A-A' OF LF-03 BIOREACTOR

Bioreactor Demonstration
Altus AFB, Oklahoma

PARSONS

Denver, Colorado

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Altus AFB and Parsons have continued to operate the system since November 2005. The bioreactor was designed to require minimal oversight and maintenance. The solar pump is the only mechanical equipment at the site. Operations consisted of periodically visiting the site to check that the pump continues to operate and to record pressure and flow data. The solar pump has operated trouble-free during the 2-year demonstration and during the biotechnology enhancement.

4.0 DEMONSTRATION RESULTS

Groundwater samples were analyzed for CAHs and a suite of geochemical indicator parameters. Groundwater analytical data for chlorinated ethene compounds for the baseline and performance monitoring events are shown in **Table 1**. Groundwater samples were collected in November 2003, prior to the start of recirculation (baseline event), and approximately 3, 7, 13, 18, and 24 months after recirculation started. Results for sampling after the initial demonstration are discussed in **Section 5.0**.

4.1 Changes in Concentrations of CAHs

Figures 3, 4, and 5 show cross-sectional views of concentrations of TCE, *cis*-1,2-DCE, and VC in groundwater, respectively, during the November 2003, June 2004, and November 2005 sampling events. **Figure 3** shows a significant reduction in concentrations of TCE from November 2003 to June 2004, which directly correlates with increased concentrations of *cis*-1,2-DCE and VC (**Figures 4 and 5**). A rebound in TCE concentrations beginning with the December 2004 sampling event is attributed to high rates of rainfall and infiltration during October and November 2004, with consequent enhanced leaching of TCE sorbed to source area soil into groundwater.

In contrast, concentrations of VC decreased in 13 of 18 wells (including the collection trench sump LS-1) from June 2004 to November 2005. This may correlate to an overall decrease in dissolved organic carbon (DOC) concentrations and an increase in oxidation-reduction potential (ORP) as a result of the influx of oxygenated water from precipitation recharge in late 2004 and a gradual depletion of the bioavailable organic carbon derived from the mulch substrate. TCE and *cis*-1,2-DCE are not as readily degraded under these less reducing conditions.

4.2 Bioreactor Efficiency

The TCE and total chlorinated ethene removal efficiency of the bioreactor is calculated using the equation:

$$E = \frac{(C_I - C_R)}{C_I} \times 100 \qquad \text{(Equation 1)}$$

where:

C_I = Influent concentration (extraction trench sump - LS-1)
C_R = Mean Concentration in bioreactor (wells SW3 and SW4)

TABLE 1
SUMMARY OF CHLORINATED ETHENES IN GROUNDWATER

Well ID (location)	Sample Date	TCE [a/] (µg/L)[b/]	1,1-DCE [a/] (µg/L)	*cis* -1,2-DCE (µg/L)	*trans* -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Ethene (µg/L)
LS-1	9-Nov-03	408	<0.625	851	13	<0.575	0.36
(sump in	17-Feb-04	44	12 J [c/]	5485 J	47	14 J	-- [d/]
extraction trench)	22-Jun-04	1,018	<5.0	4,489	41 J	466	0.65
	10-Dec-04	626	<4.4	2,487	31 J	359	1.6
	21-Apr-05	2,179	<1.76	1,730	30 J	431	2.8
	07-Nov-05	1,620	<2.20	1,280	28 J	350	2.9
	23-Jul-06	180	<2.30	999	24 J	429	4.3
	27-Jan-07	1,300	14 J	4360 J	53	316	3.6
	11-Jul-07	1,210	<9.2	3,930	50 J	246	1.9
LS-2	21-Jun-04	1,168	<2.5	3,985	48 J	698	--
(sample port in	09-Dec-04	392	<2.2	2,096	26 J	397	--
piping)	21-Apr-05	1,882	<2.2	1,621	30 J	387	--
	10-Nov-05	1,670	<2.20	1,580	31 J	303	--
WL250	24-Jun-04	5.23 J	<1.25	32 J	5.9 J	777	--
(downgradient)	09-Dec-04	3.40 J	<0.22	19	7.0 J	184 J	--
	20-Apr-05	15.9 J	<0.44	333	19 J	466	--
	16-Nov-05	6.50 J	<0.220	5.4 J	6.6 J	221	--
	23-Jul-06	8.30 J	<0.460	3.8 J	5.1 J	205	--
	26-Jan-07	3.70 J	<0.460	5.6 J	6.2 J	101	--
	11-Jul-07	2.10 J	<0.460	60	14	732	--
DW1	8-Nov-03	9,888	<12.5	169 J	<18.5	<11.5	0.07
(upgradient)	19-Feb-04	84	<0.5	337	<0.74	<0.46	--
	23-Jun-04	69 J	<2.5	2984	23 J	289	--
	08-Dec-04	4,994	<2.2	867	36 J	164	0.90
	22-Apr-05	5,547	<1.1	958	49 J	390	2.3
	09-Nov-05	4410 M [e/]	<2.2	528	32 J	252	2.50
	23-Jul-06	8,290	<23	440 J	<13.5	300 J	4.40
	26-Jan-07	804	<2.3	328	24 J	217	3.40
	11-Jul-07	1,280	<2.3	638	65	554	6.60
DW2	7-Nov-03	323	<0.313	14	<0.463	<0.288	0.89
(upgradient)	19-Feb-04	270	<0.25	88	<0.37	<0.23	--
	24-Jun-04	55	<0.05	35	1.2 J	2.0 J	--
	08-Dec-04	33	0.26 J	35	5.9 J	166	0.80
	22-Apr-05	32	<0.044	33	5.0	140	0.35
	09-Nov-05	32 M	<0.110	5.3	6.2	144	1.70
DW3	8-Nov-03	9,137	<12.5	201 J	<18.5	<11.5	0.44
(beneath reactor)	19-Feb-04	225 J	<12.5	9,777	<18.5	<11.5	0.56
	23-Jun-04	21 J	<5	5,394	50 J	1,762	5.3
	09-Dec-04	4,094	<4.4	1,434	43 J	679	3.9
	20-Apr-05	1,119	11.2 J	3,189	49 J	1,446	11,000
	08-Nov-05	7,170 M	<4.40	3,550	44 J	574	0.12
	22-Jul-06	9,740	<23.0	1,330	<13.5	325 J	21.0
	26-Jan-07	1,010	<4.60	2,880	43 J	668	36.0
	11-Jul-07	134	<2.3	1,280	40 J	1,040	40.0
DW4	8-Nov-03	381	<0.5	20 J	<0.74	<0.46	0.04
(beneath reactor)	18-Feb-04	387	<0.5	134	<0.74	<0.46	--
	22-Jun-04	287	<0.5	740	10 J	1,313	--
	07-Dec-04	2,713	8.1 J	1402	32 J	783	7.0
	22-Apr-05	783	12.3 J	2862	38 J	909	9.6
	11-Nov-05	3,990 M	15.0 J	5310	52	458	10.0

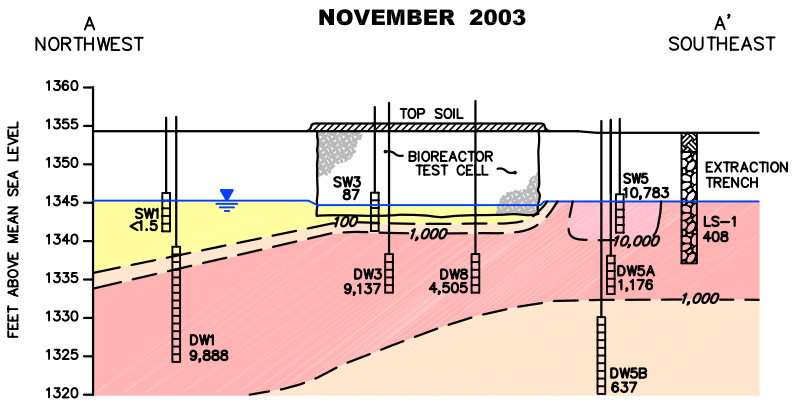
TABLE 1 (Continued)
SUMMARY OF CHLORINATED ETHENES IN GROUNDWATER

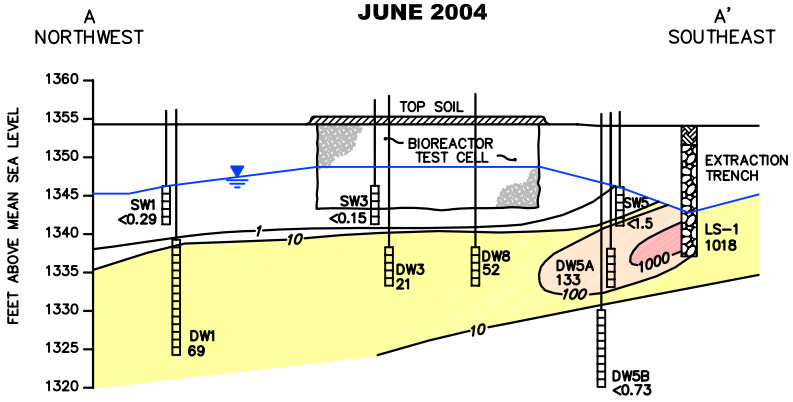
Well ID (location)	Sample Date	TCE [a/] (µg/L)[b/]	1,1-DCE [a/] (µg/L)	*cis* -1,2-DCE (µg/L)	*trans* -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Ethene (µg/L)
DW5A	7-Nov-03	1,176	<1.25	190	<1.85	<1.15	0.10
(downgradient)	18-Feb-04	182	<0.25	216	2.6 J	<0.23	0.02
	23-Jun-04	133	<0.125	128	5.8 J	146	0.49
	08-Dec-04	1,784	6.1 J	1,316	34 J	608	4.2
	19-Apr-05	1,391	<1.1	562	17 J	162	2.2
	10-Nov-05	1740 M	1.3 J	337	17	67	0.53
	22-Jul-06	380	<0.92	45	10 J	17 J	0.07
	26-Jan-07	198	<0.46	35	20	24.7	0.33
	11-Jul-07	230	<0.92	100	20 J	42.4	0.48
DW5B	7-Nov-03	637	<0.625	369	4.9	<0.575	0.08
(downgradient)	18-Feb-04	292	<0.625	752	10 J	<0.575	--
	23-Jun-04	<0.725	<0.625	3.2 J	3.2 J	397	--
	07-Dec-04	176	2.56 J	361	30	410	3.2
	19-Apr-05	35	0.67 J	87	21	177	1.3
	10-Nov-05	78 M	<0.550	128	85	278	1.40
	22-Jul-06	325	1.1 J	115	85	129	0.49
	26-Jan-07	198	<0.460	86	88	106	0.87
	11-Jul-07	205	<0.46	97	94	110	0.80
DW6A	6-Nov-03	2,197	<2.5	242	<3.7	<2.3	0.12
(downgradient)	18-Feb-04	439	<0.5	361	2.7 J	<0.46	0.10
	23-Jun-04	569	<0.625	224	<0.925	89	0.45
	09-Dec-04	396	3.2 J	644	18 J	700	3.2
	19-Apr-05	1,466	9.5 J	2,840	41	693	6.3
	19-Apr-05	1,490	11 J	2,651	41 J	677	7.30
	10-Nov-05	1510 M	5.0 J	1,640	36	296	4.20
DW6B	7-Nov-03	464	<0.5	47	<0.74	<0.46	0.05
(downgradient)	18-Feb-04	138	<0.025	9	0.10 J	<0.023	0.02
	23-Jun-04	161	<0.25	32	<0.37	<0.23	0.07
	10-Dec-04	479	<0.22	178	2.6 J	72.6	0.27
	20-Apr-05	1,039	2.09 J	522	8.6 J	107	0.95
	10-Nov-05	632 M	<0.220	200	4.1 J	40	0.36
DW7	8-Nov-03	3,190	<3.75	68 J	<5.55	<3.45	--
(beneath reactor)	18-Feb-04	160	<3.75	5,507	20 J	<3.45	--
	22-Jun-04	<4.35	<3.75	3,003	25 J	2338	--
	08-Dec-04	1,212	6.28 J	785	23 J	908	--
	22-Apr-05	28 J	<0.88	196	23 J	1197	--
	11-Nov-05	752 M	<3.30	3,690	47 J	1040	--
DW8	9-Nov-03	4,505	<5	161 J	<7.4	<4.6	--
(beneath reactor)	18-Feb-04	393	<5	5,329	25 J	<4.6	--
	22-Jun-04	52 J	<5	4,327	35 J	1290	--
	07-Dec-04	2,714	<4.4	1,303	35 J	1526	--
	22-Apr-05	3,603	<2.2	1,853	47 J	1904	--
	08-Nov-05	6,400 M	<4.40	1,780	38 J	1200	--
	22-Jul-06	6,190	<11.5	895	28 J	955	99.0
	27-Jan-07	55	<2.30	262	6.5 J	498	36.0
	11-Jul-07	386	<2.3	912	36 J	644	13.0

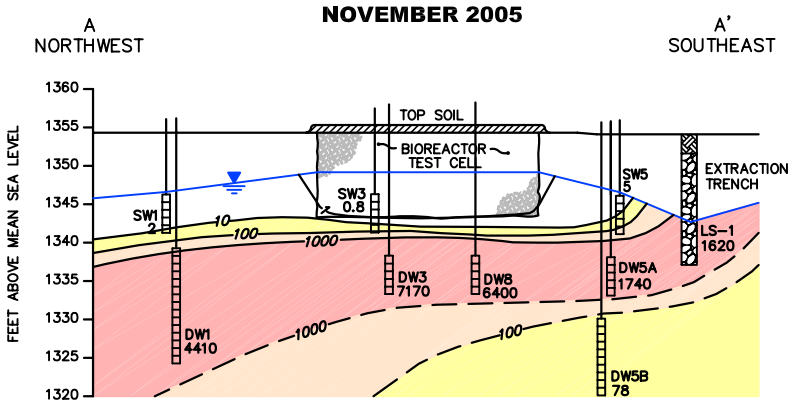
TABLE 1 (Continued)
SUMMARY OF CHLORINATED ETHENES IN GROUNDWATER

Well ID (location)	Sample Date	TCE [a/] (µg/L)[b/]	1,1-DCE [a/] (µg/L)	*cis* -1,2-DCE (µg/L)	*trans* -1,2-DCE (µg/L)	Vinyl Chloride (µg/L)	Ethene (µg/L)
SW1	8-Nov-03	<1.45	<1.25	127	<1.85	<1.15	0.07
(upgradient)	19-Feb-04	<1.45	<1.25	1,371	<1.85	<1.15	--
	24-Jun-04	<0.29	<0.25	269	3.4 j	232	--
	08-Dec-04	1.5 J	<0.22	60	4.2 J	221	1.60
	22-Apr-05	12	<0.22	136	11	363	1.20
	09-Nov-05	1.6 J	<0.220	11.9	9.2 J	374	2.60
	22-Jul-06	<0.27	<0.460	3.9 J	5.4 J	213	4.30
	26-Jan-07	1.3 J	<0.115	5.2	3.3	75	1.20
	11-Jul-07	8.7 J	1.7 J	1250	23	696	1.40
SW2	7-Nov-03	618	<0.625	76	<0.925	<0.575	0.06
(upgradient)	19-Feb-04	4.89 J	<0.625	929	6.4 J	<0.575	--
	24-Jun-04	<0.725	<0.625	455	6.2 J	291	--
	08-Dec-04	<0.775	<0.55	50	8.2 J	331	1.40
	22-Apr-05	21 J	<0.55	361	16 J	524	0.87
	09-Nov-05	2.8 J	<0.550	18 J	13 J	619	3.50
SW3	9-Nov-03	87 J	<2.5	34 J	<3.7	<2.3	9.2
(in bioreactor cell)	18-Feb-04	<0.725	<0.625	805	<0.925	3.1 J	<0.001
	22-Jun-04	<0.145	<0.125	<0.105	3.5 J	122.9	0.22
	09-Dec-04	2.9 J	<0.55	25 J	15.2 J	308.1	0.27
	20-Apr-05	5.4 J	<0.22	58	29.8	520.9	0.03
	08-Nov-05	0.75 J	<0.110	0.55 J	4.5 J	42.8	0.14
	23-Jul-06	<0.135	<0.230	0.70 J	6.1	135.0	0.46
	26-Jan-07	1.53	<0.046	7.4	2.6	22.2	0.25
	11-Jul-07	39.60	<0.23	960.0	29.8	715.0	0.68
SW4	8-Nov-03	240	<2.0	60 J	<2.96	<1.84	5.2
(in bioreactor cell)	19-Feb-04	2.4 J	<0.50	499	<0.74	3.5 J	0.04
	23-Jun-04	<0.29	<0.25	<0.21	6.0 J	265	0.21
	07-Dec-04	3.8 J	<0.22	13	10	159	0.20
	22-Apr-05	76	<0.44	312	26	482	0.50
	09-Nov-05	<0.310	<0.220	<0.390	7.4 J	114	0.42
	23-Jul-06	<0.135	<0.23	5.85	6.4	138	0.37
	20-Jan-07	0.28 J	<0.046	7.89	2.64	27.4	0.10
	11-Jul-07	20.2	<0.23	1210	32.70	748.0	0.51
SW5	6-Nov-03	10,783	<12.5	1,353	<18.5	<11.5	0.11
(downgradient)	17-Feb-04	24 J	<2.5	2,494	16 J	<2.3	0.14
	22-Jun-04	<1.45	<1.25	70	9.6 J	941	3.00
	07-Dec-04	2.1 J	<0.22	17	7.1 J	227	1.30
	18-Apr-05	47	1.5 J	611	23	487	4.20
	08-Nov-05	4.8 J	<0.550	11 J	12 J	442	4.70
	22-Jul-06	5.1 J	<0.460	4.2 J	6.6 J	304	7.20
	26-Jan-07	1.5 J	<0.460	6.1 J	7.0 J	163	3.00
	11-Jul-07	6.3 J	<0.460	320	22	708	46
SW6	6-Nov-03	14,063	<12.5	1,629	<18.5	<11.5	0.20
(downgradient)	17-Feb-04	<1.45	<1.25	1,329	10 J	159	0.20
	21-Jun-04	<0.725	<0.625	58	4.9 J	548	2.8
	09-Dec-04	6.0	<0.11	39	5.0 J	160	1.4
	19-Apr-05	14 J	<0.55	541	15 J	317	0.78
	10-Nov-05	3.5 J	<0.550	11 J	7.8 J	296	1.40

[a/] TCE = trichloroethene; DCE = dichloroethene.
[b/] µg/L = micrograms per liter.
[c/] J = estimated value.
[d/] -- = not analyzed.
[e/] M = a matrix effect was present. Concentration is estima







SCALE: HORIZONTAL 1"=25'
VERTICAL 1"=25'

LEGEND

- MULCH/SAND MIXTURE
- WATER TABLE
- 100 — CONCENTRATION CONTOUR (µg/L) (DASHED WHERE INFERRED)
- MONITORING WELL WITH SCREEN INTERVAL
- DW3 9,137 TCE CONCENTRATION (µg/L)

FIGURE 3

TCE CONCENTRATIONS CROSS-SECTION A-A'

Bioreactor Demonstration
Altus AFB, Oklahoma

PARSONS

Denver, Colorado

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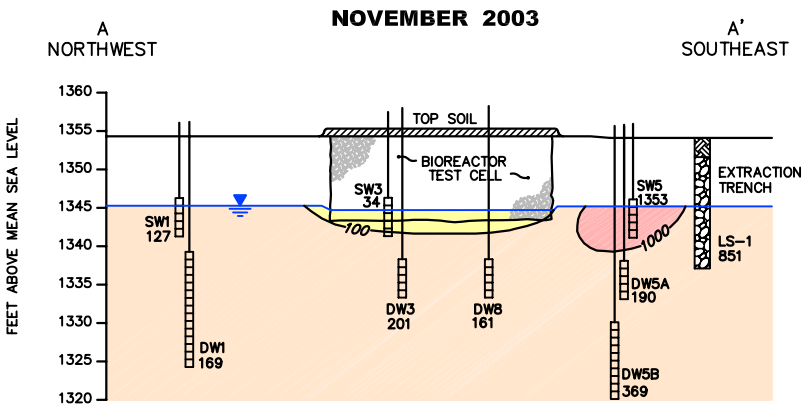

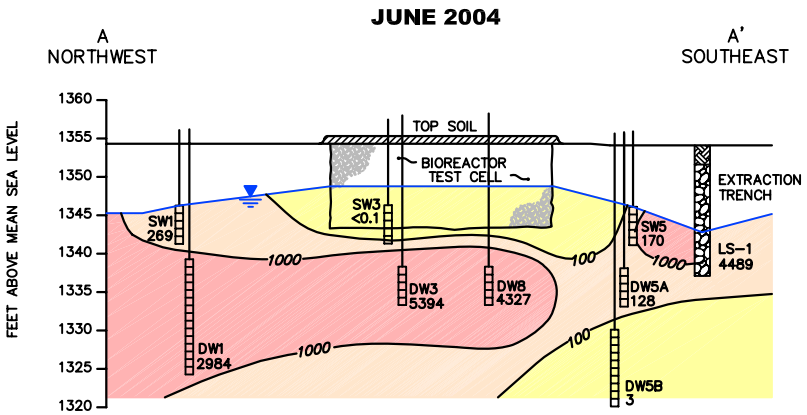

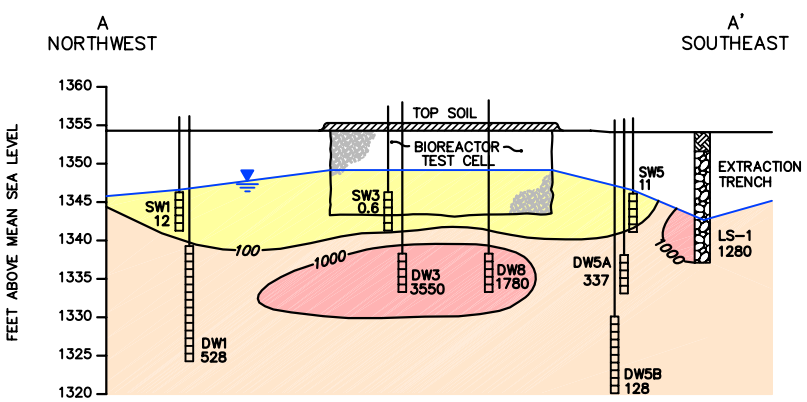

0' 25'

SCALE: HORIZONTAL 1"=25'
VERTICAL 1"=25'

LEGEND

MULCH/SAND MIXTURE

WATER TABLE

100 CONCENTRATION CONTOUR (µg/L)
(DASHED WHERE INFERRED)

MONITORING WELL WITH SCREEN INTERVAL

DW3
201 CIS-1,2-DCE CONCENTRATION (µg/L)

FIGURE 4

cis-1,2-DCE CONCENTRATIONS CROSS-SECTION A-A'

Bioreactor Demonstration
Altus AFB, Oklahoma

PARSONS
Denver, Colorado

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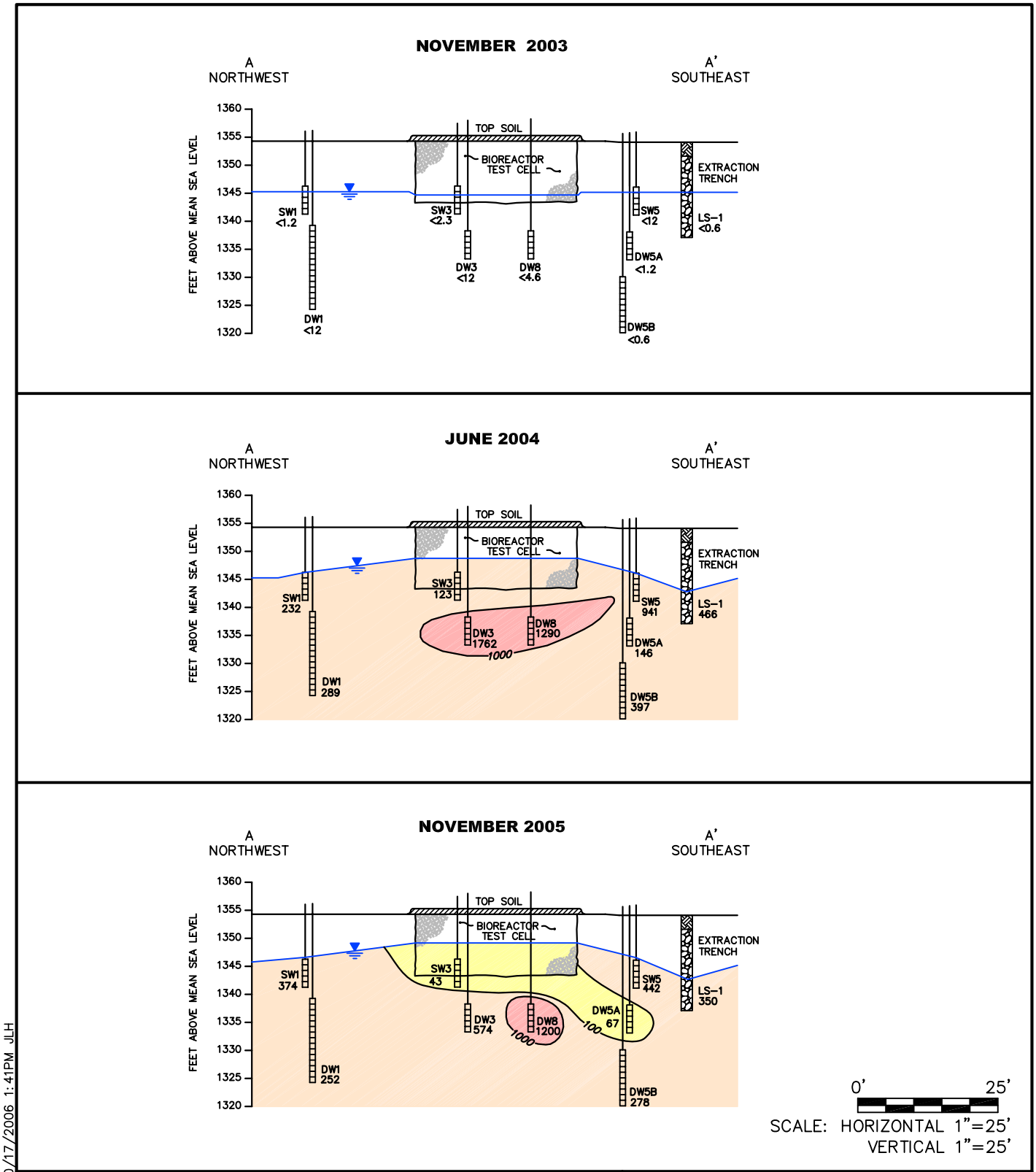

FIGURE 5

VINYL CHLORIDE CONCENTRATIONS CROSS-SECTION A-A'

Bioreactor Demonstration
Altus AFB, Oklahoma

PARSONS

Denver, Colorado

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For the five performance monitoring events from February 2004 to November 2005, the bioreactor had a 97 to 100 percent TCE removal efficiency and a total chlorinated ethene removal efficiency of 76 to 96 percent. The removal efficiencies of TCE and total chlorinated ethenes were evaluated as a primary performance metric for the pilot test. Several variables were examined to determine the cause of the inconsistent removal efficiency of the bioreactor. Variables such as changes in DOC, influent sulfate concentrations, and influent CAH concentrations do not appear to have affected the bioreactor's chlorinated ethene removal efficiency.

Changes in groundwater temperature, however, did correlate to the chlorinated ethene removal efficiency. When the average groundwater temperature in the bioreactor exceeded 20 degrees Celsius (^{o}C) the removal efficiency of total chlorinated ethenes exceeded 90 percent. Microorganisms exhibit a characteristic envelope of temperature tolerance (Chapelle, 1993). As groundwater temperature increases above the minimum growth temperature, the growth rate increases until the optimum temperature is reached. Above the optimum temperature, microorganism growth rates decline. Most bacteria present in subsurface environments are mesophiles which generally grow most efficiently from approximately 20 to 30 ^{o}C (Chapelle, 1993).

4.3 TCE Mass Removal

The rate at which TCE was at least partially dechlorinated to DCE, VC, or ethene was estimated using the difference in TCE concentrations between water entering the bioreactor via the recirculation system and groundwater samples from wells screened within the bioreactor interior. The difference in TCE concentrations was multiplied by the volume of water recirculated through the bioreactor to solve for mass of TCE removed. The estimated mass of TCE biodegraded within the bioreactor over the initial 2-year operation period was at least 6.5 lb (2.9 kilograms), or an average of approximately 3.3 lb per year (lb/yr). This is likely a lower bound to the mass of TCE degraded because this calculation does not include an estimate of the TCE removed within the bioreactor prior to the start of groundwater recirculation. Comparison of baseline TCE concentrations within the bioreactor with historical TCE concentrations measured in adjacent well WL250 indicates that substantial TCE degradation occurred in the 2 weeks between bioreactor and monitoring well installation and the baseline groundwater sampling event.

Estimating the mass of TCE removed *in situ* from the aquifer is problematic because the site is not a closed system. Increases in total molar concentrations over time within the monitored volume of the saturated zone indicate that a continuing TCE source was present in, or upgradient of, the bioreactor area during the demonstration. Groundwater geochemical data indicate that conditions conducive to reductive dechlorination of TCE extended into the shallow aquifer adjacent to and beneath the bioreactor.

4.4 Toxicity Reduction

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and RCRA remediation evaluation processes require that each candidate technology be evaluated against nine criteria, including long-term effectiveness and the reduction of contaminant toxicity, mobility, and mass over time. A common concern of reductive dechlorination technologies is the generation of toxic dechlorination products, specifically VC. These daughter products have the potential to pose an equal or greater risk to human health and the environment than the parent

compound of concern. VC is a known human carcinogen and has been assigned a federal maximum contaminant level (MCL) of 2.0 µg/L. The physiochemical properties of VC also make it more mobile in soil gas and groundwater than TCE.

Although decreases in TCE concentrations in groundwater at the LF-03 bioreactor have been accompanied by increases in intermediate dechlorination products (especially VC), calculated toxicity equivalents provide quantitative evidence that the overall toxicity of the chlorinated ethene compounds in source area groundwater has been substantially reduced. For this calculation, wells SW5 and SW6 were used as they contained the highest initial TCE concentrations, are located nearest to the historical source area well WL250, and are located on the primary groundwater flow path between the bioreactor and the extraction trench.

Toxicity equivalents are calculated by dividing each compound's concentration by its MCL (Downey *et al.*, 2006). The overall plume toxicity equivalent is the sum of the individual compound's toxicity equivalents. In this way, a given concentration of a relatively toxic compound such as VC that has a relatively low MCL will yield a higher toxicity equivalent than the same concentration of a less toxic compound such as *cis*-1,2-DCE. This approach allows the degree to which the toxicity of site contaminants has changed over time to be quantified. Based on a comparison of the November 2003 (baseline) and November 2005 (24-month monitoring event) chlorinated ethene concentrations in wells SW5 and SW6, the overall toxicity reduction achieved in the source area groundwater at the LF-03 bioreactor site ranged from 90 to 97 percent.

4.2 Groundwater Geochemistry

A primary performance objective of the technology demonstration was to increase the concentration of organic carbon in the underlying aquifer to provide a substrate for native microorganisms to grow and create optimal anaerobic biogeochemical conditions for reductive dechlorination of TCE. Anaerobic dechlorination of CAHs depends on many environmental factors including strongly anaerobic conditions, presence of fermentable substrates, generation of molecular hydrogen, and appropriate microbial populations to facilitate the reactions (AFCEE *et al*., 2004). Groundwater geochemical results reflecting these conditions are presented in **Table 2**.

Background concentrations of DOC upgradient of the bioreactor are approximately 3 to 6 milligrams per liter (mg/L). **Figure 6** shows DOC concentrations for Cross-Section A-A' during November 2003, June 2004, and November 2005. The maximum DOC concentrations were observed in the bioreactor during the baseline sampling event, after installation of the mulch but prior to start-up of recirculation. The geometric mean of DOC concentrations measured in the bioreactor was 12,410 mg/L. DOC concentrations remained greater than 20 mg/L for approximately 6 to 12 months in the deep wells beneath the bioreactor, and for almost the entire 2-year duration of the pilot test at the shallow wells directly up- and downgradient of the test cell. These concentrations are considered to be sufficient to sustain anaerobic reductive dechlorination of CAHs (USEPA, 1998).

ORP was measured to provide a relative indication of the oxidation-reduction state of groundwater at the site. Baseline ORP values from wells not impacted by the bioreactor cell suggest that relatively oxidizing conditions were present prior to installation of the test cell. The maximum baseline ORP value was 173 millivolts (mV) in deep well DW6A. The lowest ORP measured during the performance monitoring period was -372 mV in monitoring well SW4 in November 2005, screened within the bioreactor mulch.

TABLE 2
SUMMARY OF GROUNDWATER GEOCHEMICAL DATA

Well ID (location)	Sample Date	pH (SU)[a/]	Oxidation Reduction Potential (mV)[a/]	Dissolved Organic Carbon (mg/L)[a/]	Dissolved Oxygen (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Hydrogen Sulfide (mg/L)	Methane (mg/L)
LS-1	9-Nov-03	6.45	-274	18	1.14	5.05	1,600	300	5.6
(sump in	17-Feb-04	6.26	-272	-- [b/]	0.49	0.12	960	3.2	--
extraction trench)	22-Jun-04	6.83	-202	30	3.54	0.46	1,100	0.54	3.6
	10-Dec-04	7.09	-227	27	1.04	0.77	760 M [c/]	0.11	5.2
	21-Apr-05	6.50	-88	48	1.00	1.70	1,000 M	0.26	6.0
	7-Nov-05	7.48	-97	16	--	1.47	480	0.06	3.9
	23-Jul-06	6.59	-264	14	0.51	--	260	--	7.2 M
	27-Jan-07	6.63	-258	8.4	1.08	--	590 M	--	4.5
	11-Jul-07	7.92	-293	4.9	0.90	--	980	--	2.0 M
LS-2	20-Feb-04	6.49	-207	23	0.70	0.39	1,300	0.29	1.3
(sample port in	21-Jun-04	6.64	-230	--	1.31	--	--	--	--
piping)	9-Dec-04	7.13	-188	--	1.27	--	--	--	--
	21-Apr-05	6.50	-102	--	0.40	--	--	--	--
	10-Nov-05	7.29	-134	--	1.49	--	--	--	--
	11-Jul-07	8.12	-229	--	--	--	--	--	--
WL250	12-Nov-03	6.66	-159	--	1.07	--	--	--	--
(downgradient)	20-Feb-04	6.45	-253	--	1.30	--	--	--	--
	24-Jun-04	6.50	-241	--	1.72	2.0	--	1.8	--
	9-Dec-04	7.15	-153	--	1.07	--	--	--	--
	20-Apr-05	6.60	-128	--	0.70	--	--	--	--
	10-Nov-05	7.26	-165	--	0.75	9.8	--	0.09	--
	23-Jul-06	6.63	-344	--	0.37	--	--	--	--
	26-Jan-07	6.68	-176	--	3.29	--	--	--	--
	11-Jul-07	7.87	-199	--	0.90	--	--	--	--
DW1	8-Nov-03	6.88	96	3.0 J [d/]	1.28	0.35	2,000	0.60	0.0013
(upgradient)	19-Feb-04	6.73	-138	10	0.77	0.55	--	0.05	--
	23-Jun-04	6.64	-197	32	1.43	3.12	--	0.33	--
	8-Dec-04	7.08	-180	11	0.98	0.98	1,300 M	0.07	2.5
	22-Apr-05	6.60	-127	30	0.80	1.07	1,400 M	0.07	6.1
	9-Nov-05	7.48	-178	12	1.00	1.01	1,300	0.09	3.2
	23-Jul-06	6.52	-238	33	0.55	--	970	--	3.8 M
	26-Jan-07	6.61	-192	4.4	1.58	--	1,500	--	2.4
	11-Jul-07	7.26	10	7.1	1.00	--	1,000	--	7.5 M
DW2	7-Nov-03	7.07	78	5.8	0.60	0.28	2,300	1.6	0.0013
(upgradient)	19-Feb-04	6.87	-4	9.0	2.05	0.02	--	0.02	--
	24-Jun-04	6.80	-63	6.8	2.03	0.03	--	0.03	--
	8-Dec-04	6.95	-151	22	1.01	0.80	1,300 M	0.40	4.0
	22-Apr-05	6.75	28	19	0.50	0.05	2,600 M	0.09	1.9
	9-Nov-05	7.61	2	7.5	1.00	0.16	2,600	0.14	2.1
DW3	8-Nov-03	6.79	-143	9.1	0.78	0.35	2,000	7.2	0.0016
(beneath reactor)	19-Feb-04	6.71	-324	20	0.75	0.33	970	4.9	4.6
	23-Jun-04	6.53	-185	43	1.36	1.26	740	0.23	7.8
	9-Dec-04	7.00	-194	24	1.38	1.91	430 M	0.19	2.8
	20-Apr-05	6.60	-121	50	1.60	2.34	820 M	0.10	6.100
	8-Nov-05	7.57	-174	12	1.00	2.02	1,000	0.08	0.016
	22-Jul-06	6.65	-287	6.4	0.38	--	1,000	--	1.5 M
	26-Jan-07	6.50	-202	16	3.79	--	740	--	4.7
	10-Jul-07	7.60	-401	16	0.50	--	250	--	11 M
DW4	8-Nov-03	6.83	-21	3.7 J	1.36	0.26	2,000	0.90	0.003
(beneath reactor)	18-Feb-04	6.36	-180	7.6	0.55	0.39	2,700	0.02	--
	22-Jun-04	6.84	-193	23	3.47	0.89	1,900	0.30	--
	7-Dec-04	6.98	-250	21 J	1.03	1.33	700 J	0.57	6.0
	22-Apr-05	6.60	-145	49	0.40	1.86	860 M	0.42	7.3
	11-Nov-05	7.33	-160	13	1.09	1.48	1,200	0.01	3.2

TABLE 2 (Continued)
SUMMARY OF GROUNDWATER GEOCHEMICAL DATA

Well ID (location)	Sample Date	pH (SU)[a/]	Oxidation Reduction Potential (mV)[a/]	Dissolved Organic Carbon (mg/L)[a/]	Dissolved Oxygen (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Hydrogen Sulfide (mg/L)	Methane (mg/L)
DW5A	7-Nov-03	6.89	90	4.6 J	0.64	0.85	2,200	2.7	0.005
(downgradient)	18-Feb-04	6.71	-73	3.8 J	0.64	0.02	2,400	0.05	0.074
	23-Jun-04	6.74	-137	9.0	1.39	0.14	2,700	0.05	0.76
	8-Dec-04	7.06	-195	22	6.31	1.01	1,200 M	0.24	4.5
	19-Apr-05	6.70	-117	25 J	0.70	0.10	1,700	2.0	1.4
	10-Nov-05	7.47	-121	5.6	0.62	0.02	1,600	0.0	0.24
	22-Jul-06	6.83	-84	8.0	0.51	--	1,900	--	0.019 M
	26-Jan-07	6.77	-224	3.4	1.76	--	2,000	--	32.0
	10-Jul-07	7.77	364	3.1	0.67	--	2,000	--	0.25 M
DW5B	7-Nov-03	6.89	98	2.4 J	0.74	0.05	2,700	0.20	0.22
(downgradient)	18-Feb-04	6.75	-151	7.2	0.71	0.59	--	0.09	--
	23-Jun-04	7.12	-348	25	1.01	0.01	2,300	5.1	--
	7-Dec-04	7.16	-261	15 J	1.13	1.16	2,100 M	6.0	2.9
	19-Apr-05	7.10	-142	29 J	0.90	0.31	1,300	1.6	0.35
	10-Nov-05	7.38	-124	8	0.81	0.75	2,000	0.03	0.87
	22-Jul-06	6.77	-190	2.9	0.34	--	1,900	--	0.21 M
	26-Jan-07	6.71	-206	3.2	3.07	--	2,000	--	0.34
	10-Jul-07	7.55	3	3.5	2.32	--	2,000	--	0.31 M
DW6A	6-Nov-03	6.91	174	6.5	3.61	--	2,200	--	0.24
(downgradient)	18-Feb-04	6.72	-35	6.9	1.80	0.78	2,300	1.9	0.18
	23-Jun-04	6.79	-43	13	1.89	0.12	2,700	0.06	0.44
	9-Dec-04	7.03	-88	26	1.08	1.15	1,300 M	0.79	2.7
	19-Apr-05	6.50	41	35 J	0.80	0.22	1,500	0.27	4.4
	10-Nov-05	7.31	-100	9.1	0.89	1.72	2,000	0.63	1.8
DW6B	7-Nov-03	7.10	84	4.9 J	1.01	1.74	1,700	3.2	0.076
(downgradient)	18-Feb-04	6.89	40	2.5 J	0.82	<0.01	2,400	0.04	0.00048
	23-Jun-04	6.93	29	5.3	2.45	0.04	2,700	0.02	0.0082
	10-Dec-04	7.36	-96	10	1.26	0.02	2,700 M	0.03	0.29
	19-Apr-05	6.80	91	17	0.50	0.02	2,700 M	0.03	0.90 J
	10-Nov-05	7.58	23	0.7 J	0.95	0	2,000	0.04	0.28
DW7	8-Nov-03	6.85	-121	5.7	0.72	1.08	2,000	2.8	--
(beneath reactor)	18-Feb-04	6.35	-265	30	0.61	<0.01	--	4.1	--
	22-Jun-04	6.79	-230	49	3.27	1.94	--	0.36	--
	8-Dec-04	6.97	-210	33	0.99	1.18	380 M	0.14	--
	22-Apr-05	6.55	-125	51	0.60	1.78	530 M	0.03	--
	9-Nov-05	7.40	-145	12	1.00	2.08	710	0.08	--
DW8	9-Nov-03	6.51	-302	180	0.65	4.58	1,800	16.6	--
(beneath reactor)	18-Feb-04	6.25	-270	23	0.60	0.54	--	2.2	--
	22-Jun-04	6.90	-233	51	3.03	0.94	--	0.60	--
	7-Dec-04	7.14	-234	40 J	1.07	1.70	680	6.0	--
	22-Apr-05	6.60	-213	58 M	0.90	1.51	560	0.57	--
	8-Nov-05	7.34	-174	21	1.00	2.16	950	0.07	--
	22-Jul-06	6.58	-303	26	0.52	--	790	--	4.7 M
	27-Jan-07	6.75	-186	32	1.23	--	230	--	5.7
	10-Jul-07	7.38	-126	20	3.15	--	370	--	8.3 M
SW1	8-Nov-03	6.80	-1.0	3.0 J	0.57	0.32	1,400	0.30	0.00061
(upgradient)	19-Feb-04	6.53	-205	110	1.20	2.43	--	0.78	--
	24-Jun-04	6.51	-238	71	2.42	1.11	--	0.56	--
	8-Dec-04	7.06	-142	42	1.02	2.95	330 M	0.26	8.8
	22-Apr-05	6.48	-110	43 M	0.50	4.65	730 M	0.02	11.0
	9-Nov-05	7.26	-149	19	1.50	6.3	250	0.38	8.9
	22-Jul-06	6.51	-256	46	0.85	--	167 M	--	9.6 M
	26-Jan-07	6.52	-201	24	1.99	--	160	--	11.0
	11-Jul-07	7.62	-140	15	--	--	540	--	14.0 M

TABLE 2 (Continued)
SUMMARY OF GROUNDWATER GEOCHEMICAL DATA

Well ID (location)	Sample Date	pH (SU)[a/]	Oxidation Reduction Potential (mV)[a/]	Dissolved Organic Carbon (mg/L)[a/]	Dissolved Oxygen (mg/L)	Ferrous Iron (mg/L)	Sulfate (mg/L)	Hydrogen Sulfide (mg/L)	Methane (mg/L)
SW2	7-Nov-03	6.78	-7	3.7 J	0.78	0.11	1,600	0.50	0.00058
(upgradient)	19-Feb-04	6.75	-210	90	3.65	0.44	--	0.49	--
	24-Jun-04	6.54	-230	52	1.85	0.93	--	0.44	--
	8-Dec-04	6.95	-165	32	1.03	3.72	380 M	0.24	8.3
	22-Apr-05	6.55	-151	47	0.60	4.91	650 M	0.05	8.4
	9-Nov-05	7.29	-147	17	1.00	4.92	1,100	0.07	9.9
SW3	9-Nov-03	5.44	-165	14,000	0.53	--	3,600	--	0.015
(in bioreactor cell)	18-Feb-04	6.30	-201	190	4.35	1.19	<1	1.3	3.0
	22-Jun-04	6.86	-290	120	2.03	0.37	3.5	1.6	7.9
	9-Dec-04	7.01	-365	35	0.96	<0.01	220 M	0.15	4.7
	20-Apr-05	6.70	-353	65	1.40	0.04	460 M	0.01	0.64
	8-Nov-05	7.31	-360	32	1.00	0.09	<5	0.54	3.80
	23-Jul-06	6.77	-533	34	-0.62	--	2.2 J	--	9.90 M
	26-Jan-07	6.60	-351	160	0.00	--	200	--	12.0
	10-Jul-07	7.69	-420	25	0.50	--	150	--	15.0 M
SW4	8-Nov-03	5.55	-131	11,000	0.53	--	4,000	--	0.008
(in bioreactor cell)	19-Feb-04	6.69	-364	210	0.70	0.36	190	4.8	4.7
	23-Jun-04	6.63	-297	110	1.20	0.44	13	1.9	8.2
	7-Dec-04	7.09	-355	64 J	0.98	<0.01	320 M	11.5	12.0
	22-Apr-05	6.60	-347	62 M	1.30	0.04	470 M	7.2	8.3
	9-Nov-05	7.42	-372	32	0.80	0.02	54	0.55	8.7
	23-Jul-06	6.60	-521	26	--	--	3.2 J	--	9.2 M
	20-Jan-07	6.60	-333	250 J	0.00	--	9.0 J	--	13.0
	11-Jul-07	7.90	-419	59	0.80	--	77	--	13.0 M
SW5	6-Nov-03	7.01	25	3,200 J	0.93	0.10	2,000	0.50	0.0038
(downgradient)	17-Feb-04	6.19	-234	120	1.10	0.54	76	3.0	9.1
	22-Jun-04	6.69	-257	75	2.20	0.35	290	1.9	7.4
	7-Dec-04	7.06	-309	43 J	0.99	<0.01	260	130	7.4
	18-Apr-05	6.60	-251	38 M	1.40	0.97	550	0.04	10.0
	8-Nov-05	7.50	-189	21	1.00	0.81	340	0.36	9.6
	22-Jul-06	6.68	-359	22	0.28	--	220	--	8.3 M
	26-Jan-07	6.69	-218	20	1.07	--	46	--	13.0
	10-Jul-07	7.67	-346	17	0.90	--	310	--	13.0 M
SW6	6-Nov-03	6.92	110	6.1	0.76	0.68	1,800	0.00	0.06
(downgradient)	17-Feb-04	6.24	-203	140	1.23	2.15	<1	1.4	5.4
	21-Jun-04	6.70	-241	70	1.30	2.42	140	1.7	7.0
	9-Dec-04	7.00	-304	48	0.95	0.38	180 M	3.2	3.8
	19-Apr-05	6.50	-160	55 J	1.50	2.15	460	0.30	7.0
	10-Nov-05	7.34	-196	21	0.64	0.08	220	0.09	6.2
WL250	12-Nov-03	6.66	-159	--	1.07	--	--	--	--
(downgradient)	20-Feb-04	6.45	-253	--	1.30	--	--	--	--
	24-Jun-04	6.50	-241	--	1.72	2.00	--	1.8	--
	9-Dec-04	7.15	-153	--	1.07	--	--	--	--
	20-Apr-05	6.60	-128	--	0.70	--	--	--	--
	10-Nov-05	7.26	-165	--	0.75	9.75	--	0.09	--
	23-Jul-06	6.63	-344	--	0.37	--	--	--	--
	26-Jan-07	6.68	-176	--	3.29	--	--	--	--
	11-Jul-07	7.87	-199	--	0.90	--	--	--	--

[a/] ^{o}C = degrees Celsius, mg/L = milligrams per liter, mV = millivolts, SU = standard pH units

[b/] -- = not analyzed.

[c/] M = a matrix effect was present. Concentration is estimated.

[d/] J = estimated value.

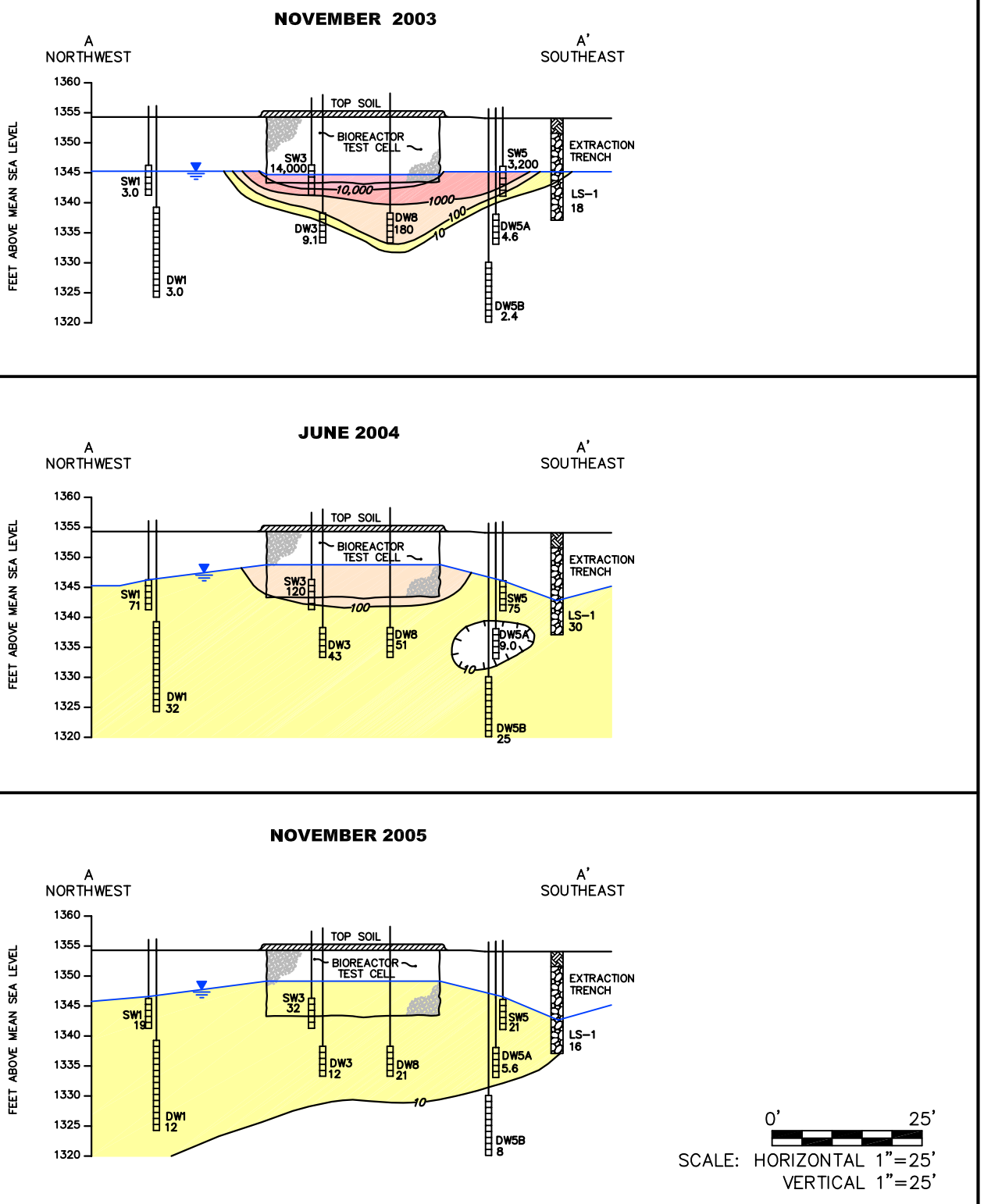

FIGURE 6

DISSOLVED ORGANIC CARBON CONCENTRATIONS CROSS-SECTION A-A'

Bioreactor Demonstration
Altus AFB, Oklahoma

PARSONS

Denver, Colorado

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Dissolved oxygen (DO) concentrations measured during performance monitoring were typically in the range of 0.5 to 1.0 mg/L. Nitrate concentrations throughout the bioreactor site were generally in the range of less than 1.0 mg/L from the start of the demonstration. Baseline ferrous iron concentrations in the aquifer were less than 1.0 mg/L and increased to a maximum of 9.8 mg/L in well WL250, located between the bioreactor and the extraction trench. Elevated ferrous iron concentrations indicate that the biologically meditated process of ferric iron reduction was occurring.

Naturally high sulfate concentrations in the aquifer at the LF-03 site hindered the ability of the bioreactor to completely deplete sulfate concentrations in groundwater underlying the bioreactor. The geometric mean sulfate concentration measured in the deep wells screened adjacent to and beneath the bioreactor was 2,074 mg/L, and likely represents a background sulfate concentration unaffected by the test cell. Sulfate concentrations decreased rapidly to less than 20 mg/L within the bioreactor and in the wells adjacent to the bioreactor by the first performance monitoring event in February 2004.

The geometric mean sulfate concentration within the test cell decreased rapidly from a high of 3,795 mg/L in November 2003 to 14 mg/L in February 2004 and 7.0 mg/L in April 2004, corresponding to a decrease of over 99 percent. At the shallow wells adjacent to the bioreactor, a geometric mean sulfate concentration of less than 20 mg/L was measured during the first performance monitoring event in February 2004, but rebounded to 201 to 589 mg/L during the subsequent events. Bioreactor influent concentrations of sulfate generally decreased over time. The lowest influent sulfate concentration was 480 mg/L, measured during the final performance monitoring event in November 2005.

The presence of methane in groundwater indicates that the anaerobic biodegradation of organic carbon via the microbially-mediated process of methanogenesis (reduction of carbon dioxide) was occurring. The average baseline methane concentration measured at the site was 0.4 mg/L, while the overall average methane concentration measured during performance monitoring was 4.8 mg/L. The increase in methane concentrations observed during performance monitoring indicates that methanogenesis was occurring and that groundwater was highly reducing, and therefore conducive to reductive dechlorination of CAHs.

5.0 BIOTECHNOLOGY ENHANCEMENT RESULTS

Although TCE has been rapidly degraded in the bioreactor, the degradation rates for *cis*-1,2-DCE and VC have been lower, resulting in accumulation of these compounds in groundwater. Therefore, a biotechnology enhancement was implemented at the LF-03 bioreactor to further stimulate the anaerobic reductive dechlorination of DCE and VC in the existing bioreactor. The work performed included 1) performing an initial condition groundwater monitoring event in July 2006, 2) augmenting the LF-03 bioreactor by addition of a commercial bioaugmentation culture containing *Dehalococcoides* ethenogenes (DHC) bacteria and supplementing the organic substrate with emulsified vegetable oil in October 2006, and 3) conducting performance groundwater monitoring events at 3 and 9 months after bioaugmentation and substrate injection.

The impact of the October 2006 biotechnology enhancement on contaminant concentrations and biogeochemical conditions (data shown in **Table 1** and **Table 2**) within and adjacent to the bioreactor was at least partially masked by influxes of surface recharge water and influx of TCE mass in late 2006/early 2007, which had a significant impact on subsurface conditions. As a

result, sampling results were not always internally consistent. However, data that indicate that the biotechnology enhancement had the desired effect include the following:

- The addition of emulsified vegetable oil to the bioreactor recirculation system caused a six-fold increase in DOC concentrations within the bioreactor; however, the increase in DOC levels appears to have been short-lived as the substrate was rapidly utilized by the existing biomass in and adjacent to the reactor.

- DCE and VC concentrations in the bioreactor interior were higher in July 2007 (final monitoring event) than at any other time during the 45-month monitoring period, most likely due to an influx of new TCE mass in precipitation recharge water and enhanced CAH dechlorination rates as a result of the substrate injection and bioaugmentation (**Table 1**).

- A large increase in ethene concentrations in shallow groundwater adjacent to the bioreactor was measured from January to July 2007, indicating that the rate of complete transformation of CAHs to non-toxic end products had increased (**Table 2**).

- From July 2006 to July 2007, total molar CAH and TCE concentrations in deeper groundwater decreased by 64% and 91%, respectively. The average total CAH removal rate within the bioreactor increased by 86% following the October 2006 enhancements (**Table 3**). This was largely due to an increase in the mass removal rate of TCE and DCE. The rate of VC removal decreased, likely due to the enhanced dechlorination of DCE to VC.

TABLE 3
MASS REMOVAL IN SOURCE AREA GROUNDWATER

	11/03 through 7/06		1/07 through 7/07		Change in Monthly Mass Removal Rate After Enhancements
	Total Mass Removed (kg) [a/]	Mass Removed per Month (kg/month)	Total Mass Removed (kg)	Mass Removed per Month (kg/month)	
Total CAHs [b/]	12.76	0.39	3.97	0.73	86%
Trichloroethene (TCE)	3.59	0.11	0.93	0.17	54%
Total Dichloroethene (DCE)	8.63	0.27	3.01	0.56	108%
Vinyl Chloride (VC)	0.54	0.02	0.03	0.00	-71%

[a/] kg = kilograms

[b/] Total CAHs = sum of trichloroethene, dichloroethene, and vinyl chloride concentrations.

- Total bacterial biomass in the bioreactor roughly doubled from July 2006 to July 2007. The absolute populations of DHC in the bioreactor influent, one bioreactor interior well, and a shallow well adjacent to the bioreactor increased from July 2006 to July 2007 by factors of 4 to nearly 8.

- Prior to January 2007, DHC strains capable of dechlorinating VC to ethene (*i.e.*, containing *vcrA* or *bvcA* reductase genes) were not detected; however, one or more of

these strains was detected in all samples collected in July 2007, indicating a general increase in bacteria that are capable of dechlorinating VC to ethene.

The ability of the bioreactor to degrade TCE all the way to ethene appears to have been enhanced by bioaugmentation. Continued periodic influxes of new TCE mass into the bioreactor system associated with periods of higher-than-normal precipitation rates tend to mask the overall reduction in CAH mass.

Perhaps a better indicator of the effectiveness of the bioreactor performance are the reductions in concentrations of CAHs observed downgradient of the bioreactor monitoring network. Data for wells on the upgradient edge of the OU-1 biowall (located approximately 200 feet downgradient of the bioreactor) show marked reductions in concentrations of TCE and *cis*-1,2-DCE after November 2003, without a significant increase of VC (see **Appendix F.2** for data at wells OU-1-1 and WL019). For example, concentrations of TCE at well OU-1-1 decreased from 5,700 µg/L in November 2003 to 239 µg/L in April 2007. Concentrations of cis-1,2-DCE similarly were reduced from 1,800 µg/L to 550 µg/L over the same period, while concentrations of VC remained low (a maximum of 12 µg/L in April 2005). These data suggest that while VC is produced in the bioreactor, concentrations of VC have not increased substantially in a downgradient direction. Rather, the bioreactor has had an overall positive impact on the OU-1 CAH plume.

6.0 TECHNOLOGY COSTS

The capital cost for constructing the Altus LF-03 recirculation pilot-scale bioreactor was approximately $56 per square foot. Because of economies of scale in excavation, materials handling and placement, and recirculation system construction, the design and construction cost for a 10,000-square foot recirculation bioreactor has been estimated at $22 per square foot and a one-acre (~44,000 square feet) bioreactor at $12 per square foot. The recirculation bioreactor is recommended for use in CAH source areas of one acre or less. At large landfills, more than one CAH source area may exist, and more than one bioreactor may be required. The potential for excavated material to require disposal as a hazardous waste should also be accounted for. Operating, maintenance, and monitoring (OM&M) costs are relatively standard for different bioreactor sizes. For example, estimated annual OM&M costs for a 1,000-square foot recirculation bioreactor (assuming semi-annual sampling) are $32,700, while annual OM&M costs for a one-acre bioreactor are estimated at $49,500.

7.0 LESSONS LEARNED - FACTORS IMPACTING BIOREACTOR PERFORMANCE

Ideal conditions for the implementation of a recirculation bioreactor system are summarized below. Not all of these conditions are required for a successful bioreactor, but these conditions will promote the most cost-effective application of the bioreactor technology. Note that this technology demonstration was focused on a landfill application; however, the bioreactor approach also can be readily implemented at other types of CAH-impacted sites.

- Dissolved CAH plume with a significant, continuing contaminant source.
- The contaminant source area can be approximately identified to be present within an area of one acre or less.

- The levels of contamination represent a long-term threat to groundwater, with source area concentrations of PCE or TCE in excess of 1.0 mg/L.

- Remedial goals cannot be met within an acceptable time frame via monitored natural attenuation (MNA). Insufficient organic matter remains in the subsurface to promote reductive dechlorination of CAHs.

- Excavation of waste material and PCE/TCE source material is a viable option at the site and the bioreactor can be constructed during the excavation backfill to further enhance the removal of PCE/TCE residuals that can not be excavated and are left behind.

- PCE/TCE plume capture can be maintained at a pumping level that is equal to or less than the allowable bioreactor recirculation/loading rate. Based on the Altus AFB LF-03 pilot bioreactor, a loading rate of 2 to 3 gallons per minute (gpm) per 1,000 square feet of bioreactor surface was sustainable. Each bioreactor will have is own optimal loading rate. The recirculation bioreactor technology may not be well-suited for thick, sandy aquifers that require the removal of large volumes of water to control the PCE/TCE source plume. Conversely, the bioreactor technology may be best suited for shallow and less permeable aquifers where smaller, more concentrated volumes of groundwater can be captured and recirculated through the bioreactor and where the vertical extent of the sustainable treatment zone is sufficient to achieve remedial goals.

- Concentrations of alternate electron acceptors in groundwater (*e.g.*, sulfate) are sufficiently low that they do not hinder complete reductive dechlorination of CAHs. At Altus AFB, the presence of sulfate in excess of 2,000 mg/L did not appear to inhibit reductive dechlorination.

The primary limitation for the technology was the ability to maintain significant levels of DOC and highly reducing conditions deeper than approximately 10 to 20 feet below the bioreactor. This limitation is site dependent and is influenced by the site-specific hydrogeology, groundwater geochemistry, and the groundwater extraction and recirculation system characteristics (*e.g.*, depth of groundwater extraction and recirculation rate).

8.0 ACKNOWLEDGEMENTS

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