Sustainability of reductive dechlorination at chlorinated solvent contaminated sites: Methods to evaluate biodegradable natural organic carbon

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Civil and Environmental Engineering

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31 October 2006
Blacksburg, VA

Keywords: Potentially Bioavailable Organic Carbon, Natural Organic Carbon, Reductive Dechlorination, Monitored Natural Attenuation

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ABSTRACT

Reductive dechlorination is a significant natural attenuation process in chloroethene-contaminated aquifers where organic carbon combined with reducing redox conditions support active dechlorinating microorganisms. At sites where natural organic carbon (NOC) associated with the aquifer matrix provides fermentable organics, the ability to measure the NOC is needed to assess the potential for the long-term sustainability of reductive dechlorination. This study focused on developing a method to measure the potentially bioavailable organic carbon (PBOC) associated with aquifer sediment.

To measure NOC and evaluate its biodegradability, liquid extraction techniques on aquifer sediment were investigated. Single extractions with different extracting solutions showed that extractable organic carbon associated with the sediment ranged from 1-38% of the total organic carbon content (TOC_s). Bioassay experiments demonstrated that 30-60% of the extractable organic carbon can be utilized by a microbial consortium. Alternating between 0.1% pyrophosphate and base solutions over multiple extractions increased the rate of removal efficiency and targeted two organic carbon pools. The result of the investigation was a laboratory method to quantify organic carbon from the aquifer matrix in terms of the PBOC. In the second part, the extractable PBOC was shown to biodegrade under anaerobic conditions, to produce H_2 at levels necessary to maintain reductive dechlorination, and to support reductive dechlorination in enrichment cultures. For the third part of the research, the difference in extractable organic carbon inside and outside of a chloroethene-contaminated plume was examined through the combination of PBOC laboratory data and field parameters. Supported by ground-water constituent data, the PBOC extraction and bioassay studies showed that less extractable organic carbon was present inside than outside of the chloroethene plume. The final part of the research investigated the distribution of PBOC extractions across six contaminated sites. PBOC extractions were directly correlated to the TOC_s, soft carbon content, and level of reductive dechlorination activity at the sites. Based on these correlations, a range

for organic carbon potentially available to subsurface microorganisms was proposed where the upper bound consisted of the soft carbon and the lower bound consisted of the PBOC.

Acknowledgements

I would like to thank my co-advisors, Dr. John Novak and Dr. Mark Widdowson, for their support throughout my studies as a Master's and PhD student. Their support during my pregnancies and after the birth of both of my children made this degree a reality.

I would like to thank Dr. Berry, Dr. Chapelle, Dr. Loganathan, and Dr. Vikesland for their support as my method slowly inched along. Their ideas and questions kept me moving forward.

I would like to thank my colleagues in the Civil and Environmental Engineering department. In Manassas, VA, the Occoquan Watershed Monitoring Laboratory faculty and staff (Dr. Grizzard, Mary Lou Daniel, Beth Biller, and Joan Wirt) provided access to instrumentation central to my degree and support for my frequent trips to NOVA. In Blacksburg, Jody Smiley and Julie Petruska were always available for advice and support. The Master's students, Kelli Mattson and Catherine Kelly, kept me focused and helped me grind sediment for countless hours. Rikke Andersen and Lauren Raup helped run experiments that were instrumental to my research. My friends and colleagues, specifically PhD Ladies Lunch, provided a wonderful support network and great baby sitters.

I would like to thank my family, Brian, Lucian and Nathaniel, for your love, support, and patience during this process. I would also like to thank my parents, Lee and Susan Veith, for not asking me when I was going to graduate.

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Chapter 1. Executive Summary

Introduction

Chloroethenes have been detected at over 50% of the National Priorities List (NPL) sites in the United States (Butler and Hayes 1999). The chloroethenes, tetrachloroethene (PCE) and trichloroethene (TCE), are used as degreasers in industrial and military facilities and are also the most commonly used chemicals in drycleaning because of their low flammability and high vapor pressures (Fetter 1999). However, through improper storage, handling, and disposal, PCE and TCE have been released into the environment and have become ubiquitous ground-water contaminants. One biogenic degradation pathway, reductive dechlorination, occurs in anaerobic environments when microorganisms reduce chloroethenes by sequentially replacing chlorine with hydrogen in the molecular structure. Microbially-mediated reductive dechlorination of PCE and TCE produces the daughter products, *cis*-1,2-dichloroethene (*cis*-1,2-DCE), *trans*-1,2-dichloroethene (*trans*-1,2-DCE), vinyl chloride (VC), ethene, and ethane (Mohn and Tiedje 1992; Maymo-Gatell et al. 1997). The ability of microorganisms to intrinsically biodegrade chlorinated solvents prompted the consideration of monitored natural attenuation (MNA) as a remediation strategy at chlorinated solvent sites (EPA 1999).

The sustainability of reductive dechlorination, and hence, the sustainability of monitored natural attenuation (MNA) as a remedial strategy at many chloroethene-contaminated sites, is dependent on a sufficient supply of reducing compounds over the time required for source and plume depletion (Chapelle et al. 2006). Microbial reductive dechlorination is sustained in ground-water systems in the presence of an ample supply of organic carbon concomitant with strongly-reducing conditions and an active dechlorinating microbial population (Bradley 2000). At sites where the fermentation of natural organic carbon (NOC) drives the reducing activity in the aquifer, the supply of an electron donor in the form of elemental hydrogen is thought to be critical in supporting contaminant biotransformation over an extended timeframe (Wiedemeier et al. 1996; Wiedemeier et al. 1998). Under anaerobic conditions, biodegradable NOC is fermented to produce hydrogen, which serves as the electron donor for dehalogenating microorganisms. Biodegradable NOC is present as dissolved organic carbon in recharge waters and the bulk

ground-water flow and is derived from renewable (soil) and non-renewable (aquifer and confining layer sediment) sources. To assess the sustainability of reductive dechlorination, it is useful to estimate the quantity of metabolizable organic carbon available in a ground-water system.

In ground-water systems, NOC can encompass a wide range of carbon compounds derived from various non-anthropogenic sources. In the evaluation of the sorptive potential of soils and sediments, Weber et al. (1992) divided NOC into two general fractions: soft carbon and hard carbon. Hard carbon contains covalent carbon crosslinked within the molecular structure and is associated with highly humified, aged aquifer material (Luthy et al. 1997). Conversely, the soft carbon consists of rubbery, amorphous material that demonstrates rapid linear partitioning of organics (Xing and Pignatello 1997; Weber et al. 2001). Whereas investigations into the properties of NOC have improved our understanding of sorption/desorption processes (Pignatello and Xing 1996; Leboeuf and Weber 2000b), the extent to which different NOC fractions biodegrade has not been extensively studied in relation to the potential reducing power contained in aquifer sediment. Furthermore, estimation of the quantity of bioavailable organic carbon, mechanisms by which this material becomes bioavailable, and its rate of utilization have not been fully investigated.

To measure NOC and evaluate its biodegradability in the context of supporting subsurface microbial populations, the first part of the research reported here investigated liquid extraction techniques on aquifer sediment. Single and multiple extraction experiments were conducted to evaluate organic carbon removal efficiencies, and the biodegradability of the extracted carbon was studied with bioassays. From these experimental results, a multiple step extraction process for the potentially bioavailable organic carbon (PBOC) associated with the sediment was proposed. In the second part, PBOC was demonstrated to biodegrade under anaerobic conditions, to produce H_2 at levels necessary to maintain reductive dechlorination, and to support reductive dechlorination in enrichment culture experiments. For the third part of the research, the difference in extractable organic carbon inside and outside of a chloroethene-contaminated plume was examined by the combination of PBOC laboratory data and field parameters. Supported by ground-water constituent data, the PBOC extractions and bioassays showed that less extractable organic carbon was present inside of the chloroethene plume than present outside of the plume. These results indicated that the PBOC method may be useful in assessing

reductive dechlorination sustainability. The final part of the research investigated the distribution of PBOC extractions and bioassays across six contaminated sites in terms of the extent of reductive dechlorination at the sites.

Investigation of extraction techniques for determining the potentially bioavailable organic carbon on aquifer sediment (Chapter 3)

The purpose of this study was to investigate extraction techniques for estimating the quantity of biodegradable organic carbon present in aquifer sediment. Single extractions with different extracting solutions (Nanopure™ water, 0.5 N sodium hydroxide, and sodium pyrophosphate) showed that extractable organic carbon associated with the sediment did not surpass the soft carbon content of the sediment. Results of single extraction bioassays demonstrated that approximately one-third of extractable organic carbon can be utilized by a microbial consortium. Repetitive extractions on aquifer sediment using either 0.1% pyrophosphate or base solutions asymptotically approached a maximum value of extractable carbon. Alternating between 0.1% pyrophosphate and base solutions increased the rate of organic carbon removal efficiency. Bioassay studies showed negligible biodegradability of the extracted organic carbon by the eighth extraction. The proposed PBOC extraction method consisted of the following sequential steps: three 24-hour extractions with 0.1% pyrophosphate, one 24-hour extraction with 0.5 N NaOH, and one 24-hour extraction with 0.1% pyrophosphate.

Anaerobic utilization of potentially bioavailable organic carbon from aquifer sediment (Chapter 4)

The objective of the second part of the research was to demonstrate that organic carbon extracted by the PBOC method can contribute to the sustainability of reductive dechlorination. Using PBOC as the sole electron donor, enrichment culture experiments exhibited reductive dechlorination of PCE through VC and established a relationship between the PBOC extractions and reductive dechlorination. Anaerobic bioassay experiments tested the biodegradability of PBOC by monitoring the decrease in aqueous total organic carbon (TOC_{aq}) and production of hydrogen gas and volatile fatty acids (VFAs) over time. The results showed that the added constraint of anaerobic conditions in comparison to the aerobic bioassays (used in chapter 3) may not be necessary to assess the biodegradability of the organic carbon removed by the extractions.

While little VFA production was observed, H_2 concentrations were observed at levels necessary to maintain reductive dechlorination. The combination of the anaerobic bioassays and the reductive dechlorination enrichment culture experiments provided lines of evidence for the utilization of PBOC extracts as a carbon source/electron donor in the subsurface and substantiated the hypothesis that PBOC extracts can support reductive dechlorination.

Evaluation of potentially bioavailable organic carbon at a chloroethene-contaminated site (Chapter 5)

The difference in extractable organic carbon inside and outside of a chloroethene-contaminated plume was investigated through combination of PBOC laboratory data and field parameters. Based on the results that PBOC supports reductive dechlorination in enrichment cultures (Chapter 4), it was hypothesized that reductive dechlorination activity would decrease the quantity of extractable PBOC within the plume. To address this hypothesis, we conducted a series of experiments comparing the sediment-bound organic carbon inside and outside of the plume. Prior to the laboratory phase, field parameters, in terms of redox constituents, were investigated to illustrate that the environment in the aquifer was amenable to reductive dechlorination. The ground-water contaminant concentrations showed that reductive dechlorination activity was active through *cis*-1,2-DCE with intermittent VC and ethene observations. After establishing that the site exhibited reductive dechlorination activity, results of PBOC extractions at shallow and deep depths showed less carbon was extracted from samples inside of the plume relative to outside of the plume. Aerobic bioassay studies demonstrated PBOC inside of the plume was less biodegradable than outside of the plume. These results further supported the assertion that reductive dechlorination activity directly decreased the quantity of extractable PBOC. Overall, these results indicated that the PBOC method may be useful in assessing reductive dechlorination sustainability.

Distribution of potentially bioavailable organic carbon extractions on aquifer sediment at chloroethene contaminated sites (Chapter 6)

Building from the previous work (Chapters 3-5), the distribution of PBOC was investigated across six contaminated sites in terms of the extent of reductive dechlorination at the sites. It was hypothesized that PBOC was correlated to the activity of reductive dechlorination at a site.

Thus, aquifer sediment was collected from six chloroethene-contaminated sites that encompassed a wide range of hydrogeology, organic carbon content, and reductive dechlorination activity. PBOC was correlated quantitatively to total organic carbon (TOC_s) and soft carbon content of the sediment and qualitatively to reductive dechlorination activity at the sites. Based on these correlations, a range for bioavailable organic carbon associated with aquifer sediment was proposed where the upper bound consisted of soft carbon and the lower bound consisted of PBOC. Both the pyrophosphate and alkali extracts contained biodegradable organic carbon at the active and minimal reductive dechlorination sites. At the sites with active reductive dechlorination, the biodegradability of the PBOC extracts was depressed, which indicated an impact on the sediment-associated organic carbon due to reductive dechlorination. Overall, variability in the results of the PBOC extractions and bioassays correlated well with reductive dechlorination activity at the sites.

Conclusions and future work

In this research, extractable organic carbon associated with aquifer sediment was investigated in the context of reductive dechlorination. An operationally defined method for estimation of potentially bioavailable organic carbon was proposed. Through a series of experiments, PBOC was shown to support reductive dechlorination directly in the laboratory and indirectly on the field scale. Moreover, correlations between PBOC extractions and field parameters showed that PBOC is related to the extent of reductive dechlorination at a site. In the next step, PBOC will be used in predictive models to evaluate the sustainability of reductive dechlorination at chloroethene-contaminated sites.

References

Bradley, P. M. (2000). "Microbial degradation of chloroethenes in groundwater systems." Hydrogeology Journal **8**(1): 104-111.

Butler, E. C. and K. F. Hayes (1999). "Kinetics of the transformation of trichloroethylene and tetrachloroethylene by iron sulfide." Environmental Science & Technology **33**(12): 2021-2027.

Chapelle, F. H., J. T. Novak, J. C. Parker, B. B. Campbell and M. A. Widdowson (in press). "A framework for assessing the sustainability of monitored natural attenuation." USGS Science Investigation Report.

EPA (1999). Final OSWER monitored natural attenuation policy. Directive 9200.4-17. O. o. S. W. a. E. Response, United States Environmental Protection Agency.

Fetter, C. W. (1999). Contaminant Hydrogeology. Upper Saddle River, NJ, Prentice Hall.

Leboeuf, E. J. and W. J. Weber (2000). "Macromolecular characteristics of natural organic matter. 2. Sorption and desorption behavior." Environmental Science & Technology **34**(17): 3632-3640.

Luthy, R. G., G. R. Aiken, M. L. Brusseau, S. D. Cunningham, P. M. Gschwend, J. J. Pignatello, M. Reinhard, S. J. Traina, W. J. Weber and J. C. Westall (1997). "Sequestration of hydrophobic organic contaminants by geosorbents." Environmental Science & Technology **31**(12): 3341-3347.

Maymo-Gatell, X., Y. T. Chien, J. M. Gossett and S. H. Zinder (1997). "Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene." Science **276**(5318): 1568-1571.

Mohn, W. W. and J. M. Tiedje (1992). "Microbial Reductive Dehalogenation." Microbiological Reviews **56**(3): 482-507.

Pignatello, J. J. and B. S. Xing (1996). "Mechanisms of slow sorption of organic chemicals to natural particles." Environmental Science & Technology **30**(1): 1-11.

Weber, W. J., E. J. Leboeuf, T. M. Young and W. L. Huang (2001). "Contaminant interactions with geosorbent organic matter: Insights drawn from polymer sciences." Water Research **35**(4): 853-868.

Weber, W. J., P. M. McGinley and L. E. Katz (1992). "A Distributed Reactivity Model for Sorption by Soils and Sediments. 1. Conceptual Basis and Equilibrium Assessments." Environmental Science & Technology **26**(10): 1955-1962.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, E. K. Gorden, J. T. Wilson, B. H. Wilson, D. H. Kambell, P. Haas, R. N. Miller, J. E. Hansen and F. H. Chapelle (1998). Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. Washington, DC, U.S. EPA.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, J. T. Wilson, D. H. Kambell, J. E. Hansen and P. Haas (1996). Overview of the technical protocol for natural attenuation of chlorinated aliphatic hydrocarbon in groundwater under development of the U.S. Air Force Center for Environmental Excellence. Proceedings of the Syposium on Natural Attenuation of Chlorinated Solvents, Washington, DC, U.S. EPA.

Xing, B. S. and J. J. Pignatello (1997). "Dual-mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter." Environmental Science & Technology **31**(3): 792-799.

Chapter 2. Literature Review

Chloroethenes

Chloroethenes have been observed at over 50% of the national priorities list sites (Butler and Hayes 1999). The chloroethenes, tetrachloroethene (PCE) and trichloroethene (TCE), are used as degreasers in industrial and military facilities as well as the main chemical in the dry cleaning process because of their low flammability and high vapor pressures (Fetter 1999). However, through improper storage, handling, and disposal PCE, TCE, and their chlorinated break down products, *cis*-1,2-dichloroethene (*cis*-1,2-DCE), *trans*-1,2-dichloroethene (*trans*-1,2-DCE), and vinyl chloride (VC), have been released into the environment and become ubiquitous ground-water contaminants. While PCE, TCE and the DCE isomers are considered probable carcinogens; VC is recognized as a known carcinogen and has been listed as a priority pollutant by the US EPA (Bradley and Chapelle 1996). Therefore, the fate of the original contaminants, PCE and/or TCE, and their progeny in the environment is of concern.

Natural Attenuation

Chloroethenes are derived from dense non-aqueous phase liquids (DNAPLs), which have a larger density than water. Hence, chloroethenes migrate through the vadose and saturated zones, and pool on confining layers (Fetter 1999). Furthermore, as the chloroethene migrates through the subsurface, ganglia of residual DNAPL remains trapped between sediment particles (Yang and McCarty 2000). These small sources of DNAPL together with the high solubility of the chloroethenes result in a dissolved phase plume (Rivett et al. 2001). Remediation strategies for chloroethenes include but are not limited to pump and treat, chemical oxidation, bioaugmentation, biostimulation, and monitored natural attenuation (MNA). Current practice in engineered remediation systems has been moving towards a treatment train approach where the source zone is actively treated to reduce the source mass with technologies such as chemical oxidation and the ground-water plume is remediated through monitored natural attenuation. While the effects of source zone treatment on the subsurface environment are integral to the

remediation strategy, the scope of this work will not address the particulars of source zone treatment but will focus on the biological aspects of the mechanisms driving the MNA of chloroethene contamination.

Monitored natural attenuation is a remediation option that utilizes the physical, chemical, and biological processes, such as dispersion, sorption, volatilization and biodegradation, to reduce the contaminant concentration (Chapelle and Bradley 1998; Wiedemeier et al. 1998). The mechanisms used by MNA for contaminant reduction can be compared by whether the mechanism is destructive or non-destructive. A destructive mechanism reduces the mass of the contamination. The destructive mechanism in MNA is biodegradation; however, abiotic processes such as hydrolysis can contribute to destructive mechanisms (Wiedemeier et al. 1998). Conversely, non-destructive mechanisms included in MNA are abiotic processes such as sorption, volatilization and dispersion. These processes reduce the concentration of the contaminant in the ground water but do not degrade the contamination. Among the mechanisms of contaminant reduction in MNA, biodegradation is thought to be the largest contributor (Wiedemeier et al. 1998).

To determine if a site is suited for MNA, the OSWER Directive 9200.4-17 proposed three lines of evidence in which to investigate the site (Wiedemeier et al. 1998). The first line of evidence addresses the historical ground-water and soil data trends at the contaminated site. The data should indicate the reduction of contaminant mass/concentration via natural attenuation processes. The majority of the contaminant reduction should be due to biodegradation rather than dilution stemming from non-destructive mechanisms such as dispersion. The second line of evidence centers on the evaluation of pertinent 'hydrogeologic and geochemical data' (Wiedemeier et al. 1998). Through the characterization of the subsurface environment with the 'hydrogeologic and geochemical data', the predominant MNA processes that affect the reduction in contamination at the site can be deduced. The final line of evidence of MNA is the evaluation of field measurements and/or the construction of microcosms to demonstrate the potential of indigenous microbiota to degrade the contaminants. Through the evaluation of each line of evidence, the ability of the site to support MNA as a viable remediation option can be assessed relative to remediation objectives associated with regulatory compliance.

Contaminant Reduction Pathways

During the later part of the 1970's and the early 1980's when the chloroethene ground-water contamination was discovered, PCE and TCE were thought to be recalcitrant (Mohn and Tiedje 1992). However, monitoring of the chloroethene plumes showed loss of PCE and TCE with concomitant increase of lesser-chloroethenes (Roberts et al. 1982; Parsons et al. 1984; Cline and Viste 1985). After a decade of research, PCE and TCE are known to degrade via multiple pathways (Debruin et al. 1992; Fetzner 1998; Bradley 2000). The most common degradation pathway, reductive dechlorination, occurs in anoxic environments and produces the lesser chloroethenes, such as *cis*-1,2-dichloroethene (*cis*-1,2-DCE), *trans*-1,2-dichloroethene (*trans*-1,2-DCE), and vinyl chloride (VC), and the innocuous end products, ethene and ethane.

For the consideration of chloroethene contaminant remediation via MNA, bioremediation has been assumed to be the primary contributor to the reduction in contaminant mass (Wiedemeier et al. 1998). However, recent research has indicated the contribution by abiotic processes to the degradation/destruction of chloroethene contamination (SERDP). In the following discussion, the various pathways for chloroethene degradation are addressed. These degradation pathways are divided into abiotic and biotic sections.

Biotic Pathways

The various biotic pathways are separated based on the redox condition of the environment and the role of the chloroethene during degradation. The degradation pathways under anoxic conditions are reductive dechlorination and anaerobic oxidation. In reductive dechlorination, the chloroethenes are used as electron acceptors where as in anaerobic oxidation the chloroethenes are electron donors. The degradation pathways in oxic environments are aerobic oxidation and aerobic cometabolism. While the chloroethenes are used as electron donors in both of these pathways, the difference between these pathways is whether the microorganisms receive energy from the degradation process. In aerobic direct oxidization, the chloroethenes are mineralized while in aerobic cometabolism the degradation is due to a fortuitous reaction with an enzyme and the microbes do not gain energy from the process. The specifics of the degradation pathways are covered in depth in various sources (Mohn and Tiedje 1992; Fetzner 1998; Chapelle 2001). Therefore, the purpose of the following discussion is to summarize biotic degradation pathways and to report on the state of recent research.

Reductive Dechlorination: It has been well established that chloroethenes can be reductively dechlorinated under anaerobic conditions (Debruin et al. 1992; Maymo-Gatell et al. 1995). During reductive dechlorination, PCE acts as an electron acceptor and can be degraded to TCE, *cis*-1,2-DCE, VC and ethene. The pathways for the reduction of the chloroethenes can be separated into two categories based on whether microorganisms derive energy from the reduction process. The first pathway is based on cometabolism where chloroethenes, such as PCE and TCE, are reduced often incompletely through a fortuitous reaction with an enzyme cofactor (Gantzer and Wackett 1991). Microorganisms such as sulfate reducers, methanogens and acetogens may cometabolize chloroethenes but they do not derive energy from the process (Gantzer and Wackett 1991; Loffler et al. 1999). The other pathway for reductive dechlorination is referred to as dehalorespiration (Fetzner 1998; Wiedemeier et al. 1998). In this process, the microorganisms derive metabolic energy by utilizing the chloroethene as an electron acceptor (Holliger and Schumacher 1994; Maymo-Gatell et al. 1997). A comparison of the degradation rates for the two reductive dechlorination pathways shows that dehalorespiration degrades chloroethenes at rates several orders of magnitude greater than cometabolism (Cabirol et al. 1998b; Cabirol et al. 1998a; Fetzner 1998; Loffler et al. 1999). Since the rate of dechlorination by cometabolism is low, the contribution of cometabolic processes to the overall degradation of the chlorinated solvents is considered negligible (Wiedemeier et al. 1998; Loffler et al. 1999). Therefore, the process of dechlororespiration will be viewed the dominant degradation pathway for reductive dechlorination in the subsurface for this project.

In dehalorespiration, the chloroethene is used as the electron acceptor for generation of metabolic energy (Holliger and Schumacher 1994; Maymo-Gatell et al. 1997). In this reaction, chlorinated solvents, such as PCE and TCE, are reduced sequentially to *cis*-1,2-DCE, VC and ethene, such that an H+ and two electrons replace one chlorine ion for each degradation reaction. The stoichiometeric reaction for PCE reduction can be written as,

$$PCE + H^+ + 2e^- \rightarrow TCE + Cl^- .$$

This process is also referred to as hydrogenolysis (Wiedemeier et al. 1998). Microorganisms capable of dehalorespiration include *Desulfitobacterium* species, *Dehalospirillum multivorans*, *Dehalobacter restricus*, and *Dehalococcoides* species (Holliger and Schumacher 1994; Fetzner

1998; He and Sanford 2002). Of these microorganisms, *Dehalococcoides ethenogenes* 195 is the only microbe known to completely reduce PCE to ethene (Maymo-Gatell et al. 1997).

As can be seen in the stoichiometeric reaction, the reductive dechlorination pathway requires an electron donor. Research has shown the predominant electron donor for dehalogenation is molecular H_2 (Holliger and Schumacher 1994; Maymo-Gatell et al. 1995; Smatlak et al. 1996; Yang and McCarty 1998). Acetate has been observed as an effective electron donor for reductive dechlorination by some dechlorinators (Krumholz et al. 1996; He et al. 2003). The production of the electron donors is attributed to the subsurface fermenting community that produce acetate and H_2 as organic carbon is fermented (McCarty and Smith 1986). Hydrogen not only supplies the dehalogenating microorganisms with their electron donors but also supports hydrogenotrophic iron-reducing, sulfate-reducing, and methanogenic microbial populations. Therefore, the various microbial populations categorized by their terminal electron acceptors (TEA), such as sulfate and chloroethenes, compete for the available electron donors.

The energy derived from the predominant terminal electron accepting process (TEAP) utilizing H_2 as an electron donor has been shown to establish a minimum H_2 threshold concentration within the system (Chapelle et al. 1996; Loffler et al. 1999). Although Lovley and Goodwin (1988) demonstrated that these H_2 threshold concentrations are dependent on physiology of the hydrogentrophic organisms opposed to the kinetics of the reactions generating and consuming H_2, the thermodynamics of the H_2 generating and consuming reactions can not be ignored. Since the conversion of organic acids to H_2 is an endothermic reaction, the levels of H_2 and other products must be kept low to drive the reaction (McCarty and Smith 1986). Thus, the synergy between the fermenting population and the hydrogentrophic organisms establishes the acceptable range for the H_2 concentration (McCarty and Smith 1986). Table 1 illustrates the ranges for hydrogen utilization as an electron donor with respect to the differing TEAPs.

Table 2-1. Ranges of Hydrogen Concentrations for TEAPs

Hydrogen Concentration (nM)	TEAP
<0.1	Denitrification
0.2 to 0.8	Iron (III) Reduction
1 to 4	Sulfate Reduction
5 to 20	Methanogenesis

Table adapted from (Chapelle et al. 1995)

It should be noted that these threshold concentrations should be applied for situations of limited electron donors. If the electron acceptor is the limiting factor, multiple TEAPs may be supported at a measured H_2 concentration (Vroblesky et al. 1996). For the dehalogenators, it has been shown that a minimum of 1 nM H_2 is required is support reductive dechlorination (EPA 1998). For a mixed culture, a 2 nM quasi-steady state hydrogen threshold was observed for reductive dechlorination (Smatlak et al. 1996). Smatlak et al. (1996) observed the type of organic acid added for H_2 production affected the predominant microbial population and suggested the use of slowly fermentable substrate to support reductive dechlorination. Subsequent research by Yang and McCarty (1998) has also shown that dechlorinators are most efficient under a steady supply of H_2 at 2 nM and better scavengers of H_2 than methanogens and homoacetogens. Overall, the process of reductive dechlorination requires the presence of electron donors at concentrations amenable to supporting the dechlorinating population.

The extent of reductive dechlorination has been shown to be affected by the predominant TEAP because the degree of chlorination of the contaminant influences the type of environment required for reductive dechlorination (Bradley 2000). The highly chlorinated solvents, such as PCE and TCE can be reduced in iron reducing environments where as the lesser chlorinated compounds, DCE and VC, require sulfate-reducing and/or methanogenic conditions in order to be reduced (Maymo-Gatell et al. 1995; Smatlak et al. 1996; Chapelle and Bradley 1998). This trend of less chlorinated compounds needing more of a reduced environment for reductive dechlorination is a result of the decreased reductive potential of DCE and VC in comparison to PCE and TCE. This decreased reductive potential leads to the decrease in the rate of reductive dechlorination which allows an accumulation of *cis*-1,2-DCE and VC in the subsurface (Wiedemeier et al. 1998). Therefore, maintaining an appropriate redox potential in the ground water is thought to be the key to sustainability of reductive dechlorination.

Anaerobic Oxidation: The ability of DCE and VC to be oxidized in reducing environments has been observed only recently (Bradley and Chapelle 1996; Bradley and Chapelle 1997). Under anaerobic conditions, it has been shown that VC can be oxidized to CO_2 (Bradley and Chapelle 1996; Bradley and Chapelle 1997; Bradley and Chapelle 1998b; Bradley et al. 1998b). Bradley and Chapelle (1998b) have demonstrated the relative rates of VC mineralization under anoxic conditions ‘decrease in the order of Fe(III)-reducing conditions, sulfate-reducing

conditions and methanogenic conditions'. VC degradation has also been observed to produce methane together with CO_2 (Bradley and Chapelle 1999b; Bradley and Chapelle 1999a). The proposed pathway for the production of methane centers on the contribution of acetotrophic methanogens that generate equal quantities of methane and CO_2 from the degradation of VC with acetate as an intermediate (Bradley and Chapelle 1999b). Under Fe(III)-reducing, sulfate-reducing, and methanogenic conditions, *cis*-1,2-DCE has been observed to mineralize to CO_2 at slower rates in comparison to VC direct oxidation (Bradley and Chapelle 1997; Bradley and Chapelle 1998b). However, VC and ethene were also observed during the course of the experiments. This observation suggests that *cis*-1,2-DCE is reductively dechlorinated to VC in a rate limiting step, then VC is oxidized to CO_2. In the presence of humic acids serving as electron acceptors, Bradley et al. (1998a) showed anaerobic oxidation of *cis*-1,2-DCE under methanogenic conditions without the detection of VC and ethene. Overall, DCE and VC can degrade under anaerobic conditions through various pathways not limited to reductive dechlorination.

Aerobic Oxidation: Under aerobic conditions, laboratory studies using field sediment samples have demonstrated the ability of the indigenous microbes to mineralize DCE and VC to CO_2 (Hartmans et al. 1985; Bradley and Chapelle 1998b; Bradley and Chapelle 1998a). Research has also shown that VC can be utilized as the sole substrate for the microbial growth (Hartmans et al. 1985; Verce et al. 2000). While research has demonstrated the mineralization of DCE for energy production by aquifer sediment without the addition of a carbon substrate, laboratory studies have not shown that DCE can act as the sole carbon source (Bradley and Chapelle 2000). Therefore, although the more reduced nature of VC allows higher rates of mineralization of VC in comparison to DCE (Bradley and Chapelle 1998b; Bradley and Chapelle 1998a), both VC and DCE are susceptible to degradation via aerobic oxidation.

Impact of Anaerobic and Aerobic Oxidation: The use of DCE and VC as electron donors as well as electron acceptors in the subsurface could mitigate the potential DCE and VC accumulation via reductive dechlorination (Bradley 2000). As the chloroethene plume is reduced and moves down gradient, the resultant DCE and VC plume may enter into more oxidized redox subsurface conditions including aerobic zones. While these conditions would

impede further reductive dechlorination, an Fe(III)-reducing environment, for example, lends itself to anaerobic oxidation of DCE and VC and aerobic conditions on the edge of the plume provides an environment amenable to DCE and VC oxidization. Since the metabolites of anaerobic and aerobic oxidation are ubiquitous, the contribution of these oxidation processes to contaminant removal is difficult to measure (Chapelle 2001). Nevertheless, the potential of anaerobic and aerobic oxidation to lesson the impact of chloroethene contamination should not be disregarded.

Aerobic Cometabolism: Aerobic cometabolism of the chloroethenes, TCE, DCE and VC, has been reported by phenol-oxidizers, methanotrophs, propylene-oxidizers and nitrifying bacteria where the end product is CO_2 (Gao and Skeen 1999; Chapelle 2001). As with cometabolic reductive dechlorination, the microbe does not gain energy from the cometabolic oxidation of the chloroethene. In order to support cometabolic oxidation, the microbial population together with their required substrate must be present in the environment. Thus the use of cometabolism in the environment may not be applicable when the substrates such as phenol and methane are required to stimulate the oxidation of chloroethenes.

Abiotic Pathways

The different types of abiotic processes currently thought to contribute to chloroethene degradation include but are not limited to sorption, volatilization, and abiotic degradation. Since the scope of this research focuses on the sustainability of the biotic process, reductive dechlorination, the discussion addressing the processes of sorption, volatilization, and abiotic degradation will be brief. Furthermore, the state of knowledge on the abiotic degradation of chloroethenes is evolving and remains the subject of current research (e.g., SERDP SON-04-02).

Sorption: The sorption processes of chloroethene contamination in aquifer sediment have been investigated extensively (Pignatello and Xing 1996; Pignatello 1998; Weber et al. 2001). Most of the research evaluates the sorption of the contaminant to the subsurface media while little research addresses the desorption of aged chloroethenes in the subsurface. Nevertheless, research by Weber and Huang (1996) has demonstrated that the predominant sorption sites in the subsurface environment are associated with NOC. For more information on the nature of NOC, see the later NOC discussion. Although research has shown that NOC sorption should not be

described as a rapid linear process, the standard engineering practice in modeling sorption of subsurface contamination utilizes linear isotherms. Therefore, the contribution of sorption processes to chloroethene contaminant reduction remains an issue of current research.

Volatilization: Volatilization from the contaminant sources located in the vadose zone and in the ground water can contribute to the reduction in contaminant mass. Residual DNAPL in the vadose zone as well as dissolved contaminants at the water table can partition into the vapor phase and migrate to the surface. Through the changes in barometeric pressure, contaminants in the ground water have been shown to partition into the vapor phase (Ellerd et al. 1999). The rate of 'barometeric pumping' on the reduction in contaminant mass decreases with depth to ground water and can be affected by perturbations to the system such as a recharge event or phreatophytes.

Abiotic Degradation: Abiotic degradation of chloroethenes by metal-based minerals has been reported in the literature. For example, research has demonstrated that PCE, TCE, and 1,1-DCE can be degraded by iron sulfides (Butler and Hayes 1999; Butler and Hayes 2001). Other metal-based minerals observed to degrade chloroethenes include zinc metal, magnetite, and copper and nickel sulfides (Butler and Hayes 1999; O'Loughlin et al. 1999; Ma et al. 2001). Acetylene was detected as an end product in these degradation processes. The detection of acetylene suggests that the degradation pathway by the metal-based minerals follows a dichloroelimination transformation as opposed to sequential hydrogenolysis as in reductive dechlorination (Butler and Hayes 1999). Dichloroelimination transformation offers the potential to reduce the production of harmful intermediates such as VC. Therefore, an understanding of the geology together with the ground-water chemistry of the subsurface environment is vital for recognizing the potential for metal-based mineral contributions to chloroethene contaminant reduction.

Reductive Dechlorination Sustainability

As related in the prior discussion, chloroethene degradation can occur through several pathways that have been extensively researched. However, the sustainability of the biotic reductive dechlorination pathway, dehalorespiration, requires further investigation over the expected time frame of MNA for chloroethene-contaminated sites. Based on current knowledge,

the factors required for sustaining dehalorespiration include an active dechlorinating microbial population, maintenance of the redox environment, and a sufficient supply of electron donor (Wiedemeier et al. 1998; Bradley 2000).

The first factor required for reductive dechlorination is an active dehalogenating population. Research has indicated that for complete chloroethene degradation often a microbial consortium is required with the exception of *Dehalococcoides ethenogenes* 195 (Harkness et al. 1999; He et al. 2003). Several researchers have grouped PCE degradation together with TCE degradation and *cis*-1,2-DCE degradation together with VC degradation (Debruin et al. 1992; Rosner et al. 1997; Magnuson et al. 1998). Other researchers have observed that two *Desulfitobacterium* species demonstrate differing degradation groupings such that strain PCE1 reduces PCE to TCE where as strain PCE-S reduces PCE to *cis*-1,2-DCE (Fetzner 1998; Miller et al. 1998). With the contributions of various microbial communities to the degradation of chloroethenes, shifts in the dominant microbial communities also occur over the time frame of MNA as the chloroethenes are reduced and the predominant contaminant changes. Research by Flynn et al. (2000) showed that additions of PCE opposed to *cis*-1,2-DCE and VC selected for different microbes using a 16S rDNA-based phylogenetic technique. The ability of the dehalogenating microbial population to degrade a chloroethene can also be influenced by current or previous redox environments (Bradley 2000; Chapelle 2001). For example after exposure to O_2 over night on a shaker table, one mixed culture of dechlorinating microbes lost its ability to reduce VC to ethene (He et al. 2003). To test for dehalogenating microbes in field samples, researchers have utilized phylogenetic techniques such as clone libraries based on 16S rDNA (Fennell et al. 2001; Richardson et al. 2002; He et al. 2003). However, the observation of rDNA from dehalogenating microbes does not indicate an active microbial population. Thus, the limitations of the selected phylogenetic technique must be recognized when interpreting the results of the test.

The second factor considered necessary to support reductive dechlorination is a reduced redox environment. As stated previously, the redox environment can affect the extent of reductive dechlorination due to the various redox potentials of the chloroethenes (Smatlak et al. 1996; Chapelle and Bradley 1998). Microbial competition for electron donors as well as abiotic factors contributes to the redox potential of a site. For example, the hydrogeology at one chloroethene-contaminated site has an organic rich layer above the contaminated aquifer (Chapelle and Bradley 1998). This organic rich layer effectively removes the oxygen from the

recharge water and makes the aquifer anoxic (Chapelle and Bradley 1998). Without the presence of the organic layer, the aquifer could receive oxic recharge waters that would influence the aquifer's redox conditions. Another factor that contributes to the subsurface redox environment is the effect of the seasons on the system. During the growing season, potential recharge water often does not reach aquifer because of evapotranspiration processes driven by vegetation. Lendvay et al. (1998) observed that temporal changes in the aquifer's redox potential exhibited a negative correlation between reductive dechlorination and oxygen concentration in the aquifer.

The third factor needed to support reductive dechlorination is a sufficient supply of electron donor, which is coupled to the establishing the redox potential necessary for selecting the dehalogentors. As stated previously, hydrogen or acetate generated by organic carbon fermentation can serve as the electron donor for reductive dechlorination (Holliger and Schumacher 1994; Maymo-Gatell et al. 1995; Smatlak et al. 1996; Yang and McCarty 1998). Thus, it is the supply of organic carbon that needs to be assessed to predict the sustainability of reductive dechlorination. The organic carbon can originate from either anthropogenic inputs (Type 1) or indigenous sources (Type 2). Type 1 organic carbon sources can be further delineated based on whether the organic material is derived from non-aqueous phase liquids (NAPLs). A Type 1-A source refers to the sites where chlorinated solvents plumes are accompanied by a carbon source containing petroleum contamination or vegetable oil. The Type 1-B source includes the sites where the organic carbon originates from non-NAPL-based sources or remediation technologies that supply organic carbon (SERDP CU-1349). When the subsurface environment can sustain a dechlorinating population without allochthonous carbon additions, this carbon source is termed a Type 2 source. For the scope of this project, the sustainability of reductive dechlorination at Type 1-B and Type 2 sites will be addressed.

Electron Donor Supply

In order to address the sustainability of reductive dechlorination at sites where the primary electron donor is natural organic carbon (NOC), the bioavailability and biodegradability of NOC must be addressed. However, little is known about the biodegradability of NOC. Moreover, a large portion NOC is often viewed as recalcitrant and is not included as a potential carbon source in anaerobic environments (Field 2002). Therefore, the evaluation of the biodegradability of NOC must not only characterize the NOC but also offer a method for measuring the

biodegradable fraction of NOC. As a basis for NOC characterization the following discussion will address natural organic carbon formation and properties. Then, based on the current understanding of NOC, the potential biodegradable components of NOC will be discussed. Finally, the current methodologies for organic carbon measurement and structural compositional analysis will be examined. This discussion should set the stage for the development of the characterization of the biodegradable NOC as well as relate the difficulties in acquiring an accurate measurement of biodegradable NOC.

Organic Carbon Forms

Based on the hypothesis that NOC can serve as an electron donor for reductive dechlorination, the different forms and properties of NOC are addressed in the following discussion. One limitation is that NOC consists of the continuum of organics not discrete sets of compounds. Nevertheless, organic carbon will be divided into the following forms: biopolymers, humic substances, and kerogens and coals (Weber et al. 2001; Song et al. 2002). The division of the organic carbon forms is based on the degree of age or diagenesis of the organic carbon.

Microbial remains and plant litter supply the biopolymers that provide the basis of natural organic carbon (Kogel-Knabner 2002). After cell death, both fungi and bacteria contribute intracellular material as well as the remains of their cell walls. Intracellular material can include proteins and lipids (Stevenson 1994). The cell walls contribute polysaccaride-based components such as peptidoglucan, which is comprised of carbohydrates and amino acids. Although much of the bacterial remains appear to be easily utilized by microbes, rudimentary building blocks such as glucosamine or muramin acid have been observed in natural organic carbon extractions (Stevenson 1994). Plant litter contributes organic substances such as cellulose, lignin, tannins, chlorophyll and starch (Kogel-Knabner 2002). The plant cell wall components, such as cellulose and lignin, degrade slowly into their basic building blocks under aerobic conditions by fungi with cellulase enzymes. Bacterial degradation of cellulose and lignin is not considered significant (Stevenson 1994; Field 2002). Therefore, both cellulose and lignin persist in the environment such that humified organics can resemble their structure (Stevenson 1994; Kogel-Knabner 2002).

Humic substances are comprised of the humified biopolymer organic material and account for the majority of the natural organic carbon (Weber et al. 2001). Humic materials can be

divided into two classes based on their relative ages (Weber et al. 2001; Song et al. 2002). Soft carbon represents younger amorphous material which has the capacity to swell or expand in aqueous environments. Conversely, hard carbon consists of highly humified or condensed carbon that is unable to expand due to cross-linked bonds within the carbon macromolecule. Another method of categorizing humic substances is based on operationally defined extraction procedures. These extractions are based on the organic carbon's solubility in dilute acid and base and divide humics into three categories: humic acids, fulvic acids and humins. Humins are insoluble in dilute alkali and acidic solutions and remain bound to the mineral surfaces. Stevenson (1994) suggests that the humins remain on the mineral due to their highly condensed nature, while Weber et al. (2001) argue that the large molecular weight of the humins or complexation to the mineral surface hinder their solubility. Humic acids that precipitate from solution upon addition of acid and fulvic acids are soluble in both alkali and acidic waters (Stevenson 1994; Pignatello 1998; Weber et al. 2001). Fulvic acids contain carbonyl and alcohol function groups that help account for their hydrophilic nature. Humic acids are more aromatic than fulvic acids and contain carboxyl and phenolic functional groups (Stevenson 1994; Pignatello 1998; Weber et al. 2001).

Kerogens and coals are the oldest types of organic carbon in the soil and sediment environment. These types of organic carbon are extremely difficult to extract from the sediment due to their high degree of cross-linked carboxyl and carbonyl groups with adjacent aromatic groups (Weber et al. 2001). Diagenetically advanced kerogens and coals can even exhibit micro regions of crystallinity (Weber et al. 2001). Based on their condensed nature, kerogens and coals are unlikely to be degraded and therefore, will not be considered as possible biodegradable carbon for the scope of this research.

NOC Partitioning

In general, the effects of functional groups in the organic carbon and attractive forces on the mineral surfaces dictate whether the organic carbon sorbs to the mineral surface or remains in the aqueous phase. The low portion of clay minerals with charge together with the prevalence of ferric iron minerals and iron oxyhydroxide coatings in both oxic and anoxic environments (Straub et al. 2001) make the interactions of organic carbon with iron-based minerals of primary interest. In aqueous environments at neutral pH, the surface of the Fe(III) minerals and coatings contains amphoteric hydroxyl groups (Kummert and Stumm 1980) that can act either as a base or

acid. Since humic acids contain high quantities of carboxyl and phenolic functional groups, the charge on these groups will be negative at typical ground-water pH. The negative charge coupled with the positive charge of most iron oxides at typical ground-water pH lends to the sorption of humic acids to the mineral surfaces (McBride 1994). Humic acids with carboxylic, phenolic or amino functional groups have been postulated to interact with the surface hydroxyl groups through ion exchange, ligand exchange, hydrophobic interactions and/or hydrogen bonds (Kummert and Stumm 1980; Gu et al. 1994). The specific mechanisms for the interactions between the oxide surface and organic carbon are still unknown and under investigation through the use of methods such as attenuated total reflectance Fourier-transform infrared (AFT FTIR) spectroscopy and ^{13}C NMR (nuclear magnetic resonance) spectroscopy. Conversely, the carbonyl and alcohol function groups content of fulvic acids as well as a low molecular weight favors the partitioning of the fulvic acids to the aqueous environment (McBride 1994; Weber et al. 2001). Similarly, the biopolymers are soluble in water with the exception of the lipids and many of them contain function groups such as carboxyl groups that would favor sorption to the mineral surface.

Sorption studies of organics on iron oxides have considered pH, ionic strength, humic acid concentration and type of humic (Gu et al. 1994; Gu et al. 1995; Avena and Koopal 1998). Adsorption of humic acids on iron oxide surfaces increased with a decrease in solution pH (Lendvay et al. 1998; Oste et al. 2002). The functional groups on the humic acid dominate the sorption reactions with the iron oxide (Gu et al. 1994; Gu et al. 1995). After the sorption process to the iron oxide, the reverse process of desorption readily occurs with changes to the solution pH holding the humic acid concentration constant (Avena and Koopal 1998). Conversely, humic acids do not readily desorb from the iron oxides when the humic acid concentration is diluted at a constant pH (Gu et al. 1995; Avena and Koopal 1998). Under the conditions of constant pH and dilute humic acid concentration, the sorption/desorption process exhibits hysteretic phenomena. Avena and Koopal (1998) argue that the ability to desorb under changes in pH is due to electrostatic forces between the oxide surface and the humic acid while the observation of the desorption hysteresis under constant pH is a result of only the concentration gradient driving the humic acid desorption.

NOC-Fe(III) Interactions

The reduction of Fe(III) during microbial oxidation of an electron donor may contribute to the bioavailability of NOC to the aqueous phase and partitioning of NOC from iron oxide surfaces. In some environments, ferric iron can be found in concentrations of 10 mmol per kg dry sediment (Straub et al. 2001). At this level, iron is the largest potential terminal electron acceptor for these environments. The use of iron oxyhydroxides and chelated Fe(III) as a terminal electron acceptor has been observed by numerous researchers (Lovley et al. 1991; Roden and Zachara 1996). Recently, iron(III)-containing clays have also been shown to provide ferric iron as a TEA for bacteria (Kostka et al. 1999). Organics, specially humic acids, have been shown to aid in Fe(III) reduction by shuttling the electron from the bacteria to Fe(III) in the iron mineral (Lovley et al. 1996; Hacherl et al. 2001). However, the fate of the NOC complexed with the iron oxide has not been addressed after the use of iron as an electron acceptor. The fate of this NOC is proposed to aid in the reducing power of the subsurface environment.

Organic Carbon Biodegradability

The complex NOC macromolecules are thought to be as the precursors to the organic acids and H_2 produced by fermentation. However, the pathway from macromolecule to organic acid/H_2 is unknown. Nevertheless, researchers have observed the utilization of complex organics to support microbial activity under anaerobic conditions and recognized the catabolic process as supporting the diagenesis of organic carbon in the subsurface (Berner 1980; Malcolm 1993). Wu et al. (1998) added complex organics to a laboratory microcosm system to test organics' ability to support reductive dechlorination. The experiments examined the effects of three types of complex organic material and cane molasses on reductive dechlorination. The microcosms with low lignin content organic matter supported reductive dechlorination better than the microcosms with high lignin content organic matter. These results were expected because the lignin is regarded generally as slowly degradable under aerobic conditions and recalcitrant under anaerobic environments (Stevenson 1994; Field 2002). However, degradation of lignin oligomers and monomers occurs in methanogenic environments have been reported (Young and Frazer 1987). In the subsurface environment, McMahon and Chapelle (1991) observed elevated volatile fatty acid concentrations in organic-rich clay layers. The production of the volatile fatty acids was attributed to the fermentation of the complex NOC in the clay layers based on predicted concentrations of acetate and formate without a microbial contribution (McMahon and

Chapelle 1991; McMahon 2001; Routh et al. 2001). Liu and Suflita (1993) demonstrated the formation of acetate from syringate via O-demethylation of H_2-CO_2 dependent subsurface sediment. Since syringate represents a typical form of the aromatic portion of NOC, the stoichiometeric production of acetate from O-demethylation indicates a potential pathway for fatty acid production from NOC. From fatty acids, hydrogen can be formed via β oxidation fermentation (McCarty and Smith 1986). Although no pathway for hydrogen generation from complex organic carbon has been described, the literature indicates the potential for utilization of complex NOC through observations of intermediate compounds indicative of microbial activity in the subsurface environment and through the use of surrogate compounds that represent the building blocks of NOC.

Organic Carbon Measurement

Some of the typical general measurement techniques for organic carbon are presented in this section. The separation of NOC is operationally defined and for the scope of this research the following definitions are proposed. The particulate organic matter (POC) will refer to the organic carbon attached to the stationary aquifer sediment (not removed via centrifugation). The aqueous phase will be further differentiated between dissolved organic carbon (DOC) and total aqueous organic carbon (TAOC). The TAOC includes the DOC (<0.2 micron) and the colloidal organic material (McCarthy 1993). However, it should be noted that the measurement of the aqueous colloidal organic carbon and the POC would include the biomass organic carbon. Malcolm (1993) states that DOC should not be frozen but stored at 4°C to minimize possible coagulation of the NOC.

The following discussion is an overview of the standard methods and their limitations for organic carbon measurement. Due to the limitations of the methods, the goals of the project will lead to permutations of these methods and most likely new method development. Nevertheless, this discussion provides a foundation for the quantification of NOC and indicates potential areas of concern.

<u>High Temperature Combustion:</u> Total of carbon can be measured with the high-temperature oxidation (combustion) technique. In this procedure, the carbon is oxidized to CO_2 and the quantity of CO_2 production is measured. For aqueous samples, prior to oxidation the samples are

acidified and purged to removed carbonates from the solution. Malcolm (1993) also argues that the samples should not be acidified after filtering through a 0.45-micron filter due to the effect on fulvics and humics. Although this procedure can be applied universally to sample containing carbon, the method does not distinguish between the various types of organic carbon. If carbon measurement for specific fractions is desired, then the carbon must be separated prior to combustion. If compositional analysis of the organic carbon is desired, those procedures should be performed prior to combustion since the combustion process is a destructive technique.

Extractions: Standard procedures to remove and separate the humic substances are listed in detail in Stevenson (1994). Currently, a method to remove of the biopolymer fraction prior to the extraction of humic substance has not been standardized (Song et al. 2002). Stevenson (1994) lists HCl and H2SO4 for extraction of amino acids and sugars under the section hydrolyzable compounds. Inherent in the extraction and separation procedures is the alteration of nature of the sorbed organic carbon. Not only in the extraction of biopolymers but also in the standard humic substance extraction procedure, the soil sample is treated with HCl. This treatment can dramatically change the structure of the natural organic carbon through acid-catalyzed hydrolysis. Furthermore, in the humic substance extraction procedure, the system is exposed to alkali in the form of NaOH which can promote saponification of the organics.

Stevenson (1994) states that natural organic carbon extraction procedures, among other requirements, should not only be able to remove the organic carbon from the soil but also preserve the form of the sorbed carbon during the extraction process. Unfortunately, achieving both results is quite difficult in practice. The above-mentioned difficulties have spurred the use of mild extractants such as sodium pyrophosphate to gently coax the organic carbon from the mineral surface (Stevenson 1994). With these mild extractants, natural organic carbon is not subjected to such a harsh environment and can be better qualified and quantified. However, the condensed nature of humic substances often times limits the effectiveness of mild extractant on removing the humics from the mineral surface (Weber et al. 2001; Song et al. 2002).

Thermal Analysis: Reviews and handbooks for thermal analysis are abundant, for example (Hatakeyama and Liu 1998). Thermal analysis allows the measurement of a physical property of the sample as a function of temperature. There are several different techniques for thermal

analysis which include differential thermal analysis (DTA) and differential scanning calorimetry (DSC). In DTA, the difference in temperature for a substance in comparison to a standard is measured over a given temperature range. The results indicate the temperature difference where the changes in the sample, such as melting, occur. DSC also uses the comparison of the sample to a reference material. However, DSC generates the difference in heat capacities as a function of temperature. In the field of NOC, Leboeuf and Weber (2000) utilized DSC for determination of glass transition temperatures for various types of organic carbon.

Organic Carbon Compositional Analysis

The following discussion summarizes several possible methods for NOC compositional analysis. The theory of the analytical techniques will not be discussed explicitly. Rather, the discussion will look at the application of the analytical technique to aid in the understanding of the molecular structure of the NOC such as alkane, alkene, and aromatic composition. Since NOC is a continuum of organics and not a discrete set of compounds, the extent of NOC qualification is limited. Therefore, the type of structural composition for each following technique will be addressed with the purpose of comparing samples to learn how the NOC changed over the coarse of an experiment.

Chromatography: Both gas and high pressure liquid chromatography can be used to assess the alkane and alkene length in NOC. One research project used the GC to examine the difference between two types of humins with fatty acid methyl ether (FAME) analysis (Rice 1991). The FAME procedure separates the long chain fatty acids in a sample after a derivatization procedure. Since chromatography can also measure the quantity of the compounds, the change in fatty acid concentration in a NOC sample would indicate microbial activity (Chefetz et al. 2002). Another technique, tetramethlyammonium hydroixde (TMAH) thermochemolysis-gas chromatography (GC)/ mass spectrometery (MS), has been used to elucidate macromolecule structural information (Chefetz et al. 2002). The lignin-derived, non-lignin aromatic, FAME, and dicarboxylic acid demethyl ester (DAME) compounds can be assessed with this procedure. These techniques both produce chromatograms that relate the relative abundance of the types of organic compounds in the sample. For example, the comparison of the same sample before and after exposure to microbial degradation could suggest the preferential utilization of specific types of compounds through fermentation.

Nuclear Magnetic Resonance: A comprehensive summary solid-state 13C-NMR theory is available (Knicker and Nanny 1997). Solid-state 13C-NMR produces spectra of the given sample of organic carbon. The peaks in the spectra can be correlated to the various types of functional groups found in the NOC. Specifically, 13C-NMR can relate the 'existence and relative quantities of aliphatic and aromatic constituents, carboxyl/ester groups, phenols, methoxyl groups' (Clapp et al. 1993; Kogel-Knabner 2002). The presence of carbohydrates and their derivatives can also be detected (Clapp et al. 1993). However, the location of the functionalities on the molecular structure cannot be interpreted from this method (Clapp et al. 1993). Through a comparison of different spectra, changes in functional groups can be observed (Kogel-Knabner 2002). Similarly to chromatographic analysis, the comparison of the same sample before and after exposure to microbial degradation could suggest the preferential utilization of specific functional groups through fermentation.

Models for Reductive Dechlorination

Currently, no model has been shown to adequately address the issue of reductive dechlorination sustainability at chlorinated solvent sites. Models are available that address specific aspects of the problem such as the biotransformation, electron donor production and redox zonation. On the field scale, two examples of numerical models that include reductive dechlorination are RT3D and SEAM3D (Clement 1997; Waddill and Widdowson 1998; Widdowson and Waddill 1998). Both models are finite difference based and built on MT3DMS and MODFLOW-2000 (Zheng and Wang 1999). RT3D offers the ability to model the biodegradation in an aquifer under either aerobic or anaerobic conditions. However, these conditions are not subject to change over time. SEAM3D expands the aquifer zonation to include each TEAP. The spatial and temporal distribution of the redox zones depends on the quantity of the terminal electron acceptors. SEAM3D also offers the capability of including direct oxidation and aerobic cometabolism into the degradation of the chlorinated solvent plume. Currently neither model incorporates the electron-donor/chloroethene interactions when predicting dechlorination. At the laboratory scale, mechanistic models can rigorously address the process of reductive dechlorination. For example, Fennell and Gossett (1998) developed a model to predict the degradation of the chloroethenes based on Michaelis Menten-type kinetics

and the interconnected nature of electron donor type, supply and competition for reductive dechlorination. Although the model developed by Fennell and Gossett (1998) incorporates electron-donor/chloroethene interactions, the model may not be applicable or practical over large spatial and temporal scales.

Organic Carbon/H_2

Throughout the literature, approaches to modeling organic carbon consumption vary in the degree of complexity. The first approach is the rigorous method taken by Fennell and Gossett (1998). In the model, the synergetic interactions between the hydrogen-producing and hydrogen-consuming microorganisms are explicitly modeled using Michaelis Menten-type kinetics. The incorporation of H_2 thresholds allowed their model to simulate the H_2 production for different electron donors coupled to the dominant microorganisms present in the system. Without the threshold or H_2 "ceiling" explicitly integrated into the model, the intrinsic parameters would not have corrected for the different substrate addition. Although not developed specifically for reductive dechlorination, the model by Hunter et al. (1998) divides the organic carbon pool into two parts: dissolved organic carbon (DOC) and particulate organic carbon (POC). Each part can be separated up to four groups and each group can have different reactivities. Hunter et al. (1998) simplifies the organic carbon reaction term to first order kinetics and justifies the assumption based on the low level of organic carbon in the subsurface. The reaction rates of the DOC and POC are integrated into the fate and transport model for the dissolved contaminants.

In comparison to the carbon specific methods taken by Fennell and Gossett (1998) and Hunter et al. (1998), Berner (1980) views the degradation of a carbon macromolecule on a holistic level such that a complex carbon molecule is degraded into carbon dioxide, water and/or methane. Since the mechanisms of bacterial degradation are enzyme driven, Michaelis-Menten kinetics is proposed for the degradation of the organic carbon. However, due to the oligotrophic nature of the subsurface environment, Michaelis-Menten kinetics is simplified to first order kinetics. It should be noted that the organic carbon entering the degradation equation consists of insoluble organic carbon not aqueous organic carbon. Berner does distinguish between the various carbon pools and their ease of degradation. The carbon pools can be summed to represent the total organic carbon content. Finally, Berner couples the reactions of inorganic species with the reactions of the organic carbon pool because of the reliance of inorganic species such as CO_2 as reactants in other microbial processes.

References

Avena, M. J. and L. K. Koopal (1998). "Desorption of humic acids from an iron oxide surface." Environmental Science & Technology **32**(17): 2572-2577.

Berner, R. (1980). Early diagenesis: a theoretcial approach. Princeton New Jersey, Princeton University Press.

Bradley, P. M. (2000). "Microbial degradation of chloroethenes in groundwater systems." Hydrogeology Journal **8**(1): 104-111.

Bradley, P. M. and F. H. Chapelle (1996). "Anaerobic mineralization of vinyl chloride in Fe(III)-reducing, aquifer sediments." Environmental Science & Technology **30**(6): 2084-2086.

Bradley, P. M. and F. H. Chapelle (1997). "Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions." Environmental Science & Technology **31**(9): 2692-2696.

Bradley, P. M. and F. H. Chapelle (1998a). "Effect of contaminant concentration on aerobic microbial mineralization of DCE and VC in stream-bed sediments." Environmental Science & Technology **32**(5): 553-557.

Bradley, P. M. and F. H. Chapelle (1998b). "Microbial mineralization of VC and DCE under different terminal electron accepting conditions." Anaerobe **4**(2): 81-87.

Bradley, P. M. and F. H. Chapelle (1999a). "Methane as a product of chloroethene biodegradation under methanogenic conditions." Environmental Science & Technology **33**(4): 653-656.

Bradley, P. M. and F. H. Chapelle (1999b). "Role for acetotrophic methanogens in methanogenic biodegradation of vinyl chloride." Environmental Science & Technology **33**(19): 3473-3476.

Bradley, P. M., F. H. Chapelle and D. R. Lovley (1998a). "Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene." Applied and Environmental Microbiology **64**(8): 3102-3105.

Bradley, P. M., F. H. Chapelle and J. T. Wilson (1998b). "Field and laboratory evidence for intrinsic biodegradation of vinyl chloride contamination in a Fe(III)-reducing aquifer." Journal of Contaminant Hydrology **31**(1-2): 111-127.

Butler, E. C. and K. F. Hayes (1999). "Kinetics of the transformation of trichloroethylene and tetrachloroethylene by iron sulfide." Environmental Science & Technology **33**(12): 2021-2027.

Butler, E. C. and K. F. Hayes (2001). "Factors influencing rates and products in the transformation of trichloroethylene by iron sulfide and iron metal." Environmental Science & Technology **35**(19): 3884-3891.

Cabirol, N., F. Jacob, J. Perrier, B. Fouillet and P. Chambon (1998a). "Complete degradation of high concentrations of tetrachloroethylene by a methanogenic consortium in a fixed-bed reactor." Journal of Biotechnology **62**(2): 133-141.

Cabirol, N., F. Jacob, J. Perrier, B. Fouillet and P. Chambon (1998b). "Interaction between methanogenic and sulfate-reducing microorganisms during dechlorination of a high concentration of tetrachloroethylene." Journal of General and Applied Microbiology **44**(4): 297-301.

Chapelle, F. H. (2001). Ground-Water Microbiology and Geochemistry. New York, John Wiley and Sons, Inc.

Chapelle, F. H. and P. M. Bradley (1998). "Selecting Remediation Goals by Assessing the Natural Attenuation Capacity of Groundwater Systems." Journal Bioremediation **2**: 227-238.

Chapelle, F. H., S. K. Haack, P. Adriaens, M. A. Henry and P. M. Bradley (1996). "Comparison of E(h) and H-2 measurements for delineating redox processes in a contaminated aquifer." Environmental Science & Technology **30**(12): 3565-3569.

Chapelle, F. H., P. B. McMahon, N. M. Dubrovsky, R. F. Fujii, E. T. Oaksford and D. A. Vroblesky (1995). "Deducing the Distribution of Terminal Electron-Accepting Processes in Hydrologically Diverse Groundwater Systems." Water Resources Research **31**(2): 359-371.

Chefetz, B., J. Tarchitzky, A. P. Deshmukh, P. G. Hatcher and Y. Chen (2002). "Structural characterization of soil organic matter and humic acids in particle-size fractions of an agricultural soil." Soil Science Society of America Journal **66**(1): 129-141.

Clapp, L. W., M. H. B. Hayes and R. S. Swift (1993). Isolation, Fractionation, Functionalities and Concepts of Structures of Soil Organic Macromolecules. Cambridge, United Kingdom, Royal Society of Chemistry.

Clement, T. P. (1997). RT3D (Version 1.0) A modular computer code for simulating reactive multi-species transport in 3-dimensional groundwater systems. PNNK-11720.

Cline, P. V. and D. R. Viste (1985). "Migration and Degradation Patterns of Volatile Organic-Compounds." Waste Management & Research **3**(4): 351-360.

Debruin, W. P., M. J. J. Kotterman, M. A. Posthumus, G. Schraa and A. J. B. Zehnder (1992). "Complete Biological Reductive Transformation of Tetrachloroethene to Ethane." Applied and Environmental Microbiology **58**(6): 1996-2000.

Ellerd, M. G., J. W. Massmann, D. P. Schwaegler and V. J. Rohay (1999). "Enhancements for passive vapor extraction: The Hanford study." Ground Water **37**(3): 427-437.

Fennell, D. E., A. B. Carroll, J. M. Gossett and S. H. Zinder (2001). "Assessment of indigenous reductive dechlorinating potential at a TCE-contaminated site using microcosms, polymerase chain reaction analysis, and site data." Environmental Science & Technology **35**(9): 1830-1839.

Fennell, D. E. and J. M. Gossett (1998). "Modeling the production of and competition for hydrogen in a dechlorinating culture." Environmental Science & Technology **32**(16): 2450-2460.

Fetter, C. W. (1999). Contaminant Hydrogeology. Upper Saddle River, NJ, Prentice Hall.

Fetzner, S. (1998). "Bacterial dehalogenation." Applied Microbiology and Biotechnology **50**(6): 633-657.

Field, J. A. (2002). "Limits of anaerobic biodegradation." Water Science and Technology **45**(10): 9-18.

Flynn, S. J., F. E. Loffler and J. M. Tiedje (2000). "Microbial community changes associated with a shift from reductive dechlorination of PCE to reductive dechlorination of cis-DCE and VC." Environmental Science & Technology **34**(6): 1056-1061.

Gantzer, C. J. and L. P. Wackett (1991). "Reductive Dechlorination Catalyzed by Bacterial Transition-Metal Coenzymes." Environmental Science & Technology **25**(4): 715-722.

Gao, J. W. and R. S. Skeen (1999). "Glucose-induced biodegradation of cis-dichloroethylene under aerobic conditions." Water Research **33**(12): 2789-2796.

Gu, B., L. Liang and J. F. McCarthy (1994). "Adsorption and Desorption of Natural Organic-Matter on Fe-Oxide Coated Quartz Surfaces." Abstracts of Papers of the American Chemical Society **207**: 222-ENVR.

Gu, B. H., J. Schmitt, Z. Chen, L. Y. Liang and J. F. McCarthy (1995). "Adsorption and Desorption of Different Organic-Matter Fractions on Iron-Oxide." Geochimica Et Cosmochimica Acta **59**(2): 219-229.

Hacherl, E. L., D. S. Kosson, L. Y. Young and R. M. Cowan (2001). "Measurement of Iron(III) bioavailability in pure iron oxide minerals and soils using anthraquinone-2,6-disulfonate oxidation." Environmental Science & Technology **35**(24): 4886-4893.

Harkness, M. R., A. A. Bracco, M. J. Brennan, K. A. Deweerd and J. L. Spivack (1999). "Use of bioaugmentation to stimulate complete reductive dechlorination of trichloroethene in dover soil columns." Environmental Science & Technology **33**(7): 1100-1109.

Hartmans, S., J. A. M. Debont, J. Tramper and K. Luyben (1985). "Bacterial-Degradation of Vinyl-Chloride." Biotechnology Letters **7**(6): 383-388.

Hatakeyama, T. and Z. Liu (1998). Handbook of thermal analysis. England, John Wiley and Sons, Ltd.

He, J. Z., Y. B. Sung, M. E. Dollhopf, B. Z. Fathepure, J. M. Tiedje and F. E. Loffier (2003). "Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites. (vol 36, pg 3945, 2002)." Environmental Science & Technology **37**(4): 816-816.

He, Q. and R. A. Sanford (2002). "Induction characteristics of reductive dehalogenation in the ortho-halophenol-respiring bacterium, Anaeromyxobacter dehalogenans." Biodegradation **13**(5): 307-316.

Holliger, C. and W. Schumacher (1994). "Reductive Dehalogenation as a Respiratory Process." Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **66**(1-3): 239-246.

Hunter, K. S., Y. F. Wang and P. Van Cappellen (1998). "Kinetic modeling of microbially-driven redox chemistry of subsurface environments: coupling transport, microbial metabolism and geochemistry." Journal of Hydrology **209**(1-4): 53-80.

Knicker, H. and M. A. Nanny (1997). Basic theory and background. London, Oxford City Press.

Kogel-Knabner, I. (2002). "The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter." Soil Biology & Biochemistry **34**(2): 139-162.

Kostka, J. E., E. Haefele, R. Viehweger and J. W. Stucki (1999). "Respiration and dissolution of iron(III) containing clay minerals by bacteria." Environmental Science & Technology **33**(18): 3127-3133.

Krumholz, L. R., R. Sharp and S. S. Fishbain (1996). "A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation." Applied and Environmental Microbiology **62**(11): 4108-4113.

Kummert, R. and W. Stumm (1980). "The Surface Complexation of Organic-Acids on Hydrous Gamma-Al2o3." Journal of Colloid and Interface Science **75**(2): 373-385.

Leboeuf, E. J. and W. J. Weber (2000). "Macromolecular characteristics of natural organic matter. 1. Insights from glass transition and enthalpic relaxation behavior." Environmental Science & Technology **34**(17): 3623-3631.

Lendvay, J. M., S. M. Dean and P. Adriaens (1998). "Temporal and spatial trends in biogeochemical conditions at a groundwater-surface water interface: Implications for natural bioattenuation." Environmental Science & Technology **32**(22): 3472-3478.

Liu, S. and J. M. Suflita (1993). "H2-Co2-Dependent Anaerobic O-Demethylation Activity in Subsurface Sediments and by an Isolated Bacterium." Applied and Environmental Microbiology **59**(5): 1325-1331.

Loffler, F. E., J. M. Tiedje and R. A. Sanford (1999). "Fraction of electrons consumed in electron acceptor reduction and hydrogen thresholds as indicators of halorespiratory physiology." Applied and Environmental Microbiology **65**(9): 4049-4056.

Lovley, D. R., J. D. Coates, E. L. BluntHarris, E. J. P. Phillips and J. C. Woodward (1996). "Humic substances as electron acceptors for microbial respiration." Nature **382**(6590): 445-448.

Lovley, D. R. and S. Goodwin (1988). "Hydrogen Concentrations as an Indicator of the Predominant Terminal Electron-Accepting Reactions in Aquatic Sediments." Geochimica Et Cosmochimica Acta **52**(12): 2993-3003.

Lovley, D. R., E. J. P. Phillips and D. J. Lonergan (1991). "Enzymatic Versus Nonenzymatic Mechanisms for Fe(Iii) Reduction in Aquatic Sediments." Environmental Science & Technology **25**(6): 1062-1067.

Ma, H. Z., E. J. O'Loughlin and D. R. Burris (2001). "Factors affecting humic-nickel complex mediated seduction of trichloroethene in homogeneous aqueous solution." Environmental Science & Technology **35**(4): 717-724.

Magnuson, J. K., R. V. Stern, J. M. Gossett, S. H. Zinder and D. R. Burris (1998). "Reductive dechlorination of tetrachloroethene to ethene by two-component enzyme pathway." Applied and Environmental Microbiology **64**(4): 1270-1275.

Malcolm, R. L. (1993). Concentrations and composition for dissolved organic carbon in soils, streams, and groundwaters. Cambridge, United Kingdom, Royal Society of Chemistry.

Maymo-Gatell, X., Y. T. Chien, J. M. Gossett and S. H. Zinder (1997). "Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene." Science **276**(5318): 1568-1571.

Maymo-Gatell, X., V. Tandoi, J. M. Gossett and S. H. Zinder (1995). "Characterization of an H-2-Utilizing Enrichment Culture That Reductively Dechlorinates Tetrachloroethene to Vinyl-Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis." Applied and Environmental Microbiology **61**(11): 3928-3933.

McBride, M. B. (1994). Environmental Chemistry of Soils. New York, Oxford University Press.

McCarthy, J. F. (1993). Subsurface transport of natural organic matter: implications of contaminant mobility. Cambridge, United Kingdom, Royal Society of Chemistry.

McCarty, P. L. and D. P. Smith (1986). "Anaerobic Waste-Water Treatment .4." Environmental Science & Technology **20**(12): 1200-1206.

McMahon, P. B. (2001). "Aquifer/aquitard interfaces: mixing zones that enhance biogeochemical reactions." Hydrogeology Journal **9**(1): 34-43.

McMahon, P. B. and F. H. Chapelle (1991). "Microbial Production of Organic Acids in Aquitard Sediments and its Role in Aquifer Geochemistry." Nature **349**: 233-235.

Miller, E., G. Wohlfarth and G. Diekert (1998). "Purification and characterization of the tetrachloroethene reductive dehalogenase of strain PCE-S." Archives of Microbiology **169**(6): 497-502.

Mohn, W. W. and J. M. Tiedje (1992). "Microbial Reductive Dehalogenation." Microbiological Reviews **56**(3): 482-507.

O'Loughlin, E. J., D. R. Burris and C. A. Delcomyn (1999). "Reductive dechlorination of trichloroethene mediated by humic-metal complexes." Environmental Science & Technology **33**(7): 1145-1147.

Oste, L. A., E. J. M. Temminghoff and W. H. Van Riemsdijk (2002). "Solid-solution partitioning of organic matter in soils as influenced by an increase in pH or Ca concentration." Environmental Science & Technology **36**(2): 208-214.

Parsons, F., P. R. Wood and J. Demarco (1984). "Transformations of Tetrachloroethene and Trichloroethene in Microcosms and Groundwater." Journal American Water Works Association **76**(2): 56-59.

Pignatello, J. J. (1998). "Soil organic matter as a nanoporous sorbent of organic pollutants." Advances in Colloid and Interface Science **77**: 445-467.

Pignatello, J. J. and B. S. Xing (1996). "Mechanisms of slow sorption of organic chemicals to natural particles." Environmental Science & Technology **30**(1): 1-11.

Rice, J. A. (1991). Composition of humin in stream sediemtn and peat. Chelsea, Michigan, Lewis Publishers.

Richardson, R. E., V. K. Bhupathiraju, D. L. Song, T. A. Goulet and L. Alvarez-Cohen (2002). "Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques." Environmental Science & Technology **36**(12): 2652-2662.

Rivett, M. O., S. Feenstra and J. A. Cherry (2001). "A controlled field experiment on groundwater contamination by a multicomponent DNAPL: creation of the emplaced-source and overview of dissolved plume development." Journal of Contaminant Hydrology **49**(1-2): 111-149.

Roberts, P. V., J. Schreiner and G. D. Hopkins (1982). "Field-Study of Organic-Water Quality Changes During Groundwater Recharge in the Palo-Alto Baylands." Water Research **16**(6): 1025-1035.

Roden, E. E. and J. M. Zachara (1996). "Microbial reduction of crystalline iron(III) oxides: Influence of oxide surface area and potential for cell growth." Environmental Science & Technology **30**(5): 1618-1628.

Rosner, B. M., P. L. McCarty and A. M. Spormann (1997). "In vitro studies on reductive vinyl chloride dehalogenation by an anaerobic mixed culture." Applied and Environmental Microbiology **63**(11): 4139-4144.

Routh, J., E. L. Grossman, G. A. Ulrich and J. M. Suflita (2001). "Volatile organic acids and microbial processes in the Yegua formation, east-central Texas." Applied Geochemistry **16**(2): 183-195.

SERDP CU-1349 Integrated protocal for assessment of long-term sustainability of monitored natural attenuation of chlorinated solvent plumes.

Smatlak, C. R., J. M. Gossett and S. H. Zinder (1996). "Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture." Environmental Science & Technology **30**(9): 2850-2858.

Song, D. L., M. E. Conrad, K. S. Sorenson and L. Alvarez-Cohen (2002). "Stable carbon isotope fractionation during enhanced in situ bioremediation of trichloroethene." Environmental Science & Technology **36**(10): 2262-2268.

Stevenson, F. J. (1994). Humus Chemistry: Genesis, Composition, Reactions. NY, NY., John Wiley and Sons, Inc. .

Straub, K. L., M. Benz and B. Schink (2001). "Iron metabolism in anoxic environments at near neutral pH." Fems Microbiology Ecology **34**(3): 181-186.

Verce, M. F., R. L. Ulrich and D. L. Freedman (2000). "Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions." Applied and Environmental Microbiology **66**(8): 3535-3542.

Vroblesky, D. A., P. M. Bradley and F. H. Chapelle (1996). "Influence of electron donor on the minimum sulfate concentration required for sulfate reduction in a petroleum hydrocarbon-contaminated aquifer." Environmental Science & Technology **30**(4): 1377-1381.

Waddill, D. W. and M. A. Widdowson (1998). "Three-dimensional model for subsurface transport and biodegradation." Journal of Environmental Engineering-Asce **124**(4): 336-344.

Weber, W. J. and W. L. Huang (1996). "A distributed reactivity model for sorption by soils and sediments .4. Intraparticle heterogeneity and phase-distribution relationships under nonequilibrium conditions." Environmental Science & Technology **30**(3): 881-888.

Weber, W. J., E. J. Leboeuf, T. M. Young and W. L. Huang (2001). "Contaminant interactions with geosorbent organic matter: Insights drawn from polymer sciences." Water Research **35**(4): 853-868.

Widdowson, M. A. and D. W. Waddill (1998). Modeling intrinsic bioremediation of chlorinated solvents in groundwater. On site and In-situ Bioremedation, San Diego, CA, Battelle Press.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, E. K. Gorden, J. T. Wilson, B. H. Wilson, D. H. Kambell, P. Haas, R. N. Miller, J. E. Hansen and F. H. Chapelle (1998). Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. Washington, DC, U.S. EPA.

Wu, W. M., J. Nye, M. K. Jain and R. F. Hickey (1998). "Anaerobic dechlorination of trichloroethylene (TCE) to ethylene using complex organic materials." Water Research **32**(5): 1445-1454.

Yang, Y. R. and P. L. McCarty (1998). "Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture." Environmental Science & Technology **32**(22): 3591-3597.

Yang, Y. R. and P. L. McCarty (2000). "Biologically enhanced dissolution of tetrachloroethene DNAPL." Environmental Science & Technology **34**(14): 2979-2984.

Young, L. Y. and A. C. Frazer (1987). "The Fate of Lignin and Lignin-Derived Compounds in Anaerobic Environments." Geomicrobiology Journal **5**(3-4): 261-293.

Zheng, C. and P. P. Wang (1999). MT3DMS: A modular three-dimensional transport model for simulation of advection, dispersion, and chemical reactions of contaminants in ground water systems. Vicksburg, MS, U.S. Army Engineer Research and Development Center.

Chapter 3. Investigation of extraction techniques for determining the potentially bioavailable organic carbon on aquifer sediment

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Abstract

Extraction techniques to estimate the quantity of biodegradable organic carbon present in aquifer sediment were investigated. The investigation consisted of three parts, beginning with an evaluation of the carbon removal efficiency of different extracting solutions: Nanopure™ water, sodium hydroxide (0.5 N), and sodium pyrophosphate (0.1, 0.5, and 1.0%). Bioassay experiments were conducted using extracted aqueous carbon inoculated with a mixed culture of microorganisms and monitoring carbon utilization over time. The third component of the study consisted of repetitive extractions to examine the long-term extractability and relative biodegradability of organic carbon associated with sediments. The results of single extraction experiments showed that 1-38% of organic carbon associated with surface water and aquifer sediments was extracted depending on the extracting solution. Persulfate oxidation experiments produced soft carbon contents that ranged from 44-80% of organic carbon associated with the sediments. Biodegradability studies demonstrated that 30-40% of extracted organic carbon biodegraded over 45 days by soil microorganisms. Multiple extraction experiments illustrated the ability of the extracting solutions to target different pools of organic carbon and demonstrated that extracted organic carbon asymptotically approached maximum values below the sediments' soft carbon content. Biodegradability of extracted carbon from the multiple extractions decreased over the course of the multiple extractions. Based on multiple extractions and bioassay experiments, an extraction method for operationally-defined potentially bioavailable organic carbon was proposed. The intended application of this work is to estimate the quantity of sediment-associated natural organic carbon that provides fermentable organics capable of supporting reductive dechlorination at chloroethene-contaminated sites.

Introduction

The sustainability of reductive dechlorination, and hence, the sustainability of monitored natural attenuation (MNA) as a remedial strategy at many chloroethene-contaminated sites, is dependent on a sufficient supply of reducing compounds over the time required for source and plume depletion (Chapelle et al. 2006). Microbial reductive dechlorination is sustained in ground-water systems in the presence of an ample supply of organic carbon concomitant with strongly-reducing conditions and an active dechlorinating microbial population (Bradley 2000). At sites where the fermentation of natural organic carbon (NOC) drives the reducing activity in the aquifer, the supply of an electron donor in the form of elemental hydrogen is thought to be critical in supporting contaminant biotransformation over an extended timeframe (Wiedemeier et al. 1996; Wiedemeier et al. 1998). Under anaerobic conditions, biodegradable NOC is fermented to produce hydrogen, which serves as the electron donor for dehalogenating microorganisms. Biodegradable NOC is present as dissolved organic carbon in recharge waters and the bulk ground-water flow and is derived from renewable (soil) and non-renewable (aquifer and confining layer sediment) sources. To assess the sustainability of reductive dechlorination, it is useful to estimate the quantity of metabolizable organic carbon available in a ground-water system.

In ground-water systems, NOC can encompass a wide range of carbon compounds derived from various non-anthropogenic sources. In the evaluation of the sorptive potential of soils and sediments, Weber et al. (1992) divided NOC into two general fractions: soft carbon and hard carbon. Hard carbon contains covalent carbon crosslinked within the molecular structure and is associated with highly humified, aged aquifer material (Luthy et al. 1997). Conversely, soft carbon consists of rubbery, amorphous material that demonstrates rapid linear partitioning of organics (Xing and Pignatello 1997; Weber et al. 2001). Whereas investigations into the properties of NOC have improved our understanding of sorption/desorption processes (Pignatello and Xing 1996; Leboeuf and Weber 2000), the extent to which different NOC fractions biodegrade has not been extensively studied in relation to the potential reducing power contained in aquifer sediment. Furthermore, estimation of the quantity of bioavailable organic carbon, mechanisms by which this material becomes bioavailable, and its rate of utilization have not been fully investigated.

Techniques for quantifying organic carbon in sediment include weight loss on ignition (WLOI), elemental analysis, carbon oxidation, and chemical extraction (Ball et al. 1990). The WLOI and elemental analysis methods quantify carbon in the sediment through high temperature combustion. In WLOI tests, the difference in sample weight before and after combustion is attributed to organic matter oxidation with approximately 50% coming from organic carbon (Schwarzenbach et al. 2003). In elemental analysis, carbon is oxidized to CO_2, and the quantity of CO_2 is measured. Even though high temperature combustion procedures can be applied universally to samples containing carbon, these methods do not distinguish between the various types of organic carbon and do not address biodegradability of NOC. If the measurement of specific carbon fractions is desired with high temperature combustion, then the carbon must be separated prior to combustion. Oxidation of organic carbon by chemical oxidants can provide a means to quantify fractions of the organic carbon associated with the sediment without pre-treatment. For example, Weber et al. (1992) operationally defined soft organic carbon as the organic carbon oxidized with potassium persulfate. However, carbon oxidation is a destructive technique and does not allow for quantification of the rate and extent of biodegradation of this organic carbon. Pressurized extractions such as supercritical fluid extraction (pure CO_2) and pressurized liquid extraction (solvent based) target specific organic contaminants for removal from the sediment matrix (Hawthorne et al. 2000). By utilizing high pressures and solvents, pressurized extractions are not designed to quantify the total amount of NOC associated with the sediment and therefore may not be appropriate for extracting the bulk NOC from the sediment matrix.

A common laboratory technique for removing NOC from sediment is through use of a chemical (liquid) extraction (Stevenson 1994). Extractions enable evaluation of the composition, structure, formation, and utilization of NOC. However, a concern with organic carbon extraction and separation procedures is alteration of the organic carbon associated with the sediment. Some extraction procedures pre-treat the soil sample with acid (HCl) that can alter the structure of the NOC through acid-catalyzed hydrolysis. Furthermore in the humic substance extraction procedure, the system is exposed to alkali (NaOH), which can promote saponification of the organics. Stevenson (1994) states that NOC extraction procedures, among other requirements, should not only be able to remove the organic carbon from the soil but also preserve the form of the sorbed carbon during the extraction process. The difficulty in achieving both requirements

has spurred the use of mild extractants, such as sodium pyrophosphate and ethylenediaminetetraacetic acid (EDTA), to gently remove the organic carbon from the mineral surface (Yang et al. 2001). With these mild extractants, chemical alteration of the NOC is minimal and the NOC can be more accurately quantified and characterized. However, the condensed nature of humic substances often limits the effectiveness of a mild extractant on removing humics from soils and sediments (Stevenson 1994; Weber et al. 2001).

Extraction methods have been used to investigate the extent to which organic matter derived from upper horizon soils is biodegraded (Yang et al. 2001; Schnabel et al. 2002; Marschner and Kalbitz 2003). Extraction methods for removing organic carbon from soil samples use a variety of solvents such as water, pyrophosphate, and mineral salt solutions (Yang et al. 2001; Schnabel et al. 2002). The extracted solution is then inoculated with microorganisms, and the loss of dissolved organic carbon (DOC) or production of CO_2 is monitored over time (Lucena et al. 1991). A similar approach is used to quantify the biodegradability of dissolved organic matter in drinking water treatment systems (Volk and LeChevallier 2000; Escobar and Randall 2001). Marschner and Kalbitz (2003) report that the length of study and quantification of organic carbon differences (in terms of either DOC loss or CO_2 production) are the two critical parameters pertaining to organic matter biodegradability. Based on the length of the biodegradation studies, extracted organic carbon fractions can be separated into rapidly and slowly degradable fractions. However, the composition and relative quantities of these fractions are not known and differ for each soil type.

It is not known if the techniques used for soil organic matter evaluation are applicable to aquifer sediments or if these techniques are meaningful to the question of sustainability of reductive dechlorination. The impetus for this study stems from the lack of knowledge concerning the long-term sustainability of chloroethene reductive dechlorination in ground-water systems. The intended application of this work is at chloroethene-contaminated sites in which NOC associated with the sediment matrix provides fermentable organics capable of supporting reductive dechlorination, referred to as Type II sites (Wiedemeier et al. 1996). At Type II sites, reductive dechlorination is thought to be sustained by DOC in ground water derived from upgradient recharge sources and by NOC derived from aquifer sediment in contact with the aqueous phase contaminant plume.

The purpose of this study was to investigate extraction techniques for estimating the quantity of biodegradable organic carbon present in aquifer and surface water sediments. First, carbon removal efficiencies were evaluated for different extracting solutions and compared to the total organic carbon content and soft carbon content of the sediment. Next, bioassay experiments were conducted using the extracted aqueous carbon inoculated with a mixed culture of microorganisms and monitoring carbon utilization over time. The extraction and bioassay methods were adapted from research examining soil organic matter (Yang et al. 2001; Marschner and Kalbitz 2003). Then, repetitive extractions were run to examine the long-term extractability and relative biodegradability of the organic carbon associated with the sediments. Based on the results of the repetitive extraction experiments, an extraction method for an operationally-defined potentially bioavailable organic carbon was proposed.

Materials and Methods

Study Sites

Study sites were selected to provide a range of carbon content in the sediment and were chosen based on accessibility to the sites. Low- to medium-range carbon content sediment was collected from the Columbia Aquifer (AQU-Columbia) in the Atlantic Coastal Plain of Virginia. Samples were collected from the surficial aquifer at depths of 8-10 ft and 20-22 ft below land surface (henceforth referred to as shallow and deep, respectively). Surface water sediment collected from the Edisto River (SWS-Edisto) in South Carolina and from Stroubles Creek (SWS-Stroubles) in Virginia provided high carbon content samples. Surface-water sediment samples were collected using a hand auger and stored in Mason jars. Aquifer sediment samples were collected with a Geoprobe unit and stored in acetate liners. All samples were kept at 4°C until analysis.

Extraction Method

In the extraction method, 10 g of sediment, dried at 70°C and sieved through 2-mm openings, was combined with 20 mL of an extracting solution in carbon-free (acid washed, baked at 350°C for 1 hour) 40-mL glass vials. Five extracting solutions were used in the study: Nanopure™ water (pH 5), 0.1% sodium pyrophosphate (m/v, pH 8.5), 0.5% sodium pyrophosphate (pH 10), 1.0% sodium pyrophosphate (pH 11), and 0.5 N NaOH (pH 13). The Nanopure™ water was

distilled-deionized water with a resistivity of >18MΩcm. For each extracting solution, quadruplicate extractions were run. After a 24-hour extraction cycle on the rotary tumbler, the samples were centrifuged for 25 minutes at 2000 rpm (620×g) for solids separation. The supernatants were decanted and stored at 4°C until analyzed for aqueous total organic carbon (TOC_{aq}). The solid-phase organic carbon content (TOC_s) was measured before and after extraction to determine the carbon removal efficiency.

Bioassay Method

The bioassay experiments were constructed in carbon-free 40-mL glass vials using 15 mL of extracted organic carbon, 1 mL of minimal salts media (MSM) and 1 mL of a mixed soil microbial culture. The MSM contained 3.4g KH_2PO_4, 4.35g K_2HPO_4, 1.0g NH_4Cl, 150mg $MgSO_4 \bullet H_2O$, 4.5mg $MnSO_4 \bullet 4H_2O$, 0.5mg $NaMoO_4 \bullet 2H_2O$, 0.15mg H_3BO_3, 20mg $CaCl_2$, 1.5mg $ZnCl_2$, 0.5mg $CuCl_2 \bullet 2H_2O$, 1.5mg $CoCl_2 \bullet 6H_2O$, and 11mg $FeCl_2 \bullet 4H_2O$ per 100 mL. The phosphate buffered MSM brought the pH of the Nanopure™ water and pyrophosphate bioassays to 7. Bioassays constructed with base extraction solutions required addition of 100 μL of phosphoric acid (85%) to bring the bioassay pH to 7. After the construction of the aerobic live bioassays, the headspace of the vials was flushed with oxygen. Autoclaved control bioassays (two 15 minute cycles, 121°C) were constructed similarly to the live bioassays without addition of the microbial culture. Because the extractions removed microorganisms, the autoclavings were run with 48 hours separating the cycles to reduce potential microbial re-growth. After completion of the autoclave cycles, the headspace for the controls was flushed with sterile N_2 to remove trace levels of CO_2. The bioassay vials were incubated on orbital shakers at 20°C without light until sampled. Every two weeks, the headspace of the vials was sampled for CO_2. After CO_2 measurement, TOC_{aq} samples were taken. Then, the headspace of the vials was flushed with oxygen for the live bioassays and N_2 for the control bioassays.

The microorganism culture was obtained from soil through an adaptation of the method of Zuberer (1994), where 90 mL of extraction media containing 10 mL MSM and 0.1% sodium pyrophosphate (m/v) was added to 10 g of A horizon soil (Blacksburg, VA) and shaken for 5 minutes. After the sediment settled (10 minutes), the suspended mixed culture solution was transferred to a culture flask, placed on the orbital shaker, and grown at 20°C without light. The culture (10 mL) was transferred every three weeks into 90 mL of sterile extraction media

containing 0.1% sodium pyrophosphate (m/v), 10 mL MSM and 10 g soil. Prior to the addition of the culture, the extraction media was autoclaved twice (15 minutes, 121°C) to remove organics from the sediment that served as a carbon source for the culture.

Analytical Methods

Headspace CO_2 concentrations in the bioassays were determined with a gas chromatograph using a thermal conductivity detector (TCD, Shimatzu GC-14A). TOC_{aq} content in samples was analyzed via wet persulfate oxidation (Techmar Dohrmann DC-80). The TOC_s was determined by elemental analysis (ThermoFinnigan Flash EA 1112).

Persulfate Oxidation Method

The persulfate oxidation method was adapted from Cuypers et al. (2000) and provided a measure of the soft organic carbon content of the sediment (Weber et al. 1992). After the sediment was dried at 70°C and sieved through a 2-mm sieve, 5 g of sediment was combined with 0.75 g potassium persulfate and 10 mL of Nanopure™ water in carbon-free 40-mL glass vials. The vials were shaken in a rotary water bath at 70°C for three hours. Due to carbon oxidation and the production of CO_2, pressure accumulation in the headspace was released from the vials 1.5 hours into the experiment. After the oxidation step, vials were centrifuged for 10 minutes at 2000 rpm for solids separation. The supernatant was decanted and discarded (negligible TOC_{aq} concentration). The sediment pellet was washed and centrifuged twice with 20 mL of Nanopure™ water to remove residual persulfate crystals. After the saturated sediment pellet was dried at 70°C, the sediment was ground with a mortar and pestle to homogenize the sediment. Solid sediment samples were collected before and after oxidation for TOC_s analysis to determine the efficiency of persulfate carbon oxidation.

Results and Discussion

The investigation consisted of four parts, beginning with an evaluation of organic carbon removal efficiency of the different extracting solutions. Bioassay experiments followed the removal step in which extracted carbon was inoculated with a mixed culture of microorganisms, and carbon utilization was monitored over time. The third step in the investigation consisted of repetitive extractions to examine the long-term extractability and relative biodegradability of

organic carbon associated with sediments. In the final component, an extraction method for PBOC was proposed.

Prior to the evaluation of organic carbon removal efficiency, each of the four sediment samples was homogenized and characterized by quantifying solid-phase organic carbon content (TOC_s) and soft organic carbon content (Table 3-1). The TOC_s values for the two surface-water sediment samples differed by 10% with the river sediment containing more carbon than the stream sediment. The deep AQU-Columbia sediment contained a factor of ten less TOC_s than the surface water sediments (SWS-Edisto and SWS-Stroubles), and shallow AQU-Columbia sediment contained a factor of ten less TOC_s than deep AQU-Columbia sediment. Based on TOC_s values, a considerable difference in organic carbon content between surface-water sediment samples and aquifer sediment samples was shown. The low carbon content in the aquifer sediments illustrates a potential measurement constraint in developing a method to estimate potentially bioavailable organic carbon in aquifer sediment.

Soft carbon content served as the baseline estimate for the maximum removable organic carbon through liquid extraction and was hypothesized to be the primary contributor to the pool of bioavailable organic carbon. Interestingly, soft carbon content of the sediments was not a consistent percentage of TOC_s and increased as TOC_s decreased. Shallow AQU-Columbia sediment contained the lowest TOC_s and was comprised of 80% soft carbon. Similarly, the deep AQU-Columbia, SWS-Stroubles, and SWS-Edisto sediments increased in TOC_s while decreasing in percent soft carbon content (61%, 51%, and 44% soft carbon, respectively). The inverse relationship between soft carbon content and TOC_s was assumed to result from the differences in the types of carbon associated with the surface water and aquifer sediments. Furthermore, soft carbon content may also predict the ability of the extracting solutions to remove organic carbon from the sediments.

Extraction Efficiency

The quantity of extracted aqueous organic carbon (TOC_{aq}, mass of organic carbon per mass of sediment) for each sediment after exposure to the extracting solution is provided in Table 3-1 (average of triplicate or quadruplicate samples ± standard deviation). The extractions were performed in parallel to assess the relative extraction efficiency for each extracting solution. As shown in Table 3-1, Nanopure™ water released the least amount of carbon. Then, the

pyrophosphate extractions removed increasing quantities of TOC_{aq} as the concentration of pyrophosphate increased. The base solution removed the largest quantity of carbon. However, two exceptions of this trend were observed. First, the base extraction for SWS-Stroubles sediment removed 40% less organic carbon than the 1.0% pyrophosphate extraction. Second, the pyrophosphate extractions for shallow AQU-Columbia sediment removed similar quantities of carbon within the range of 20-26 mg/kg. Overall, the quantity of carbon removed by the extractions increased with the course of the Nanopure™ water, pyrophosphate, and base extractions.

Table 3-1. Total Organic Carbon (TOC_s mg C/kg sediment), Soft Carbon (mg/kg), and Extracted Aqueous Carbon Concentrations (TOC_{aq} mg/kg) for parallel Nanopure™ water, 0.1% pyrophosphate, 0.5% pyrophosphate, 1.0% pyrophosphate, and base extractions for SWS-Edisto, SWS-Stroubles, AQU-Columbia Deep, and AQU-Columbia Shallow sediment. The values for the extracted TOC_{aq} are shown with standard deviations of triplicate or quadruplicate samples.

Sediment Samples	Total Organic Carbon (mg/kg)	Soft Carbon (mg/kg)	Extracted Aqueous Carbon Concentration (mg/kg)				
			Nanopure™	0.1% Pyro	0.5% Pyro	1.0% Pyro	Base
SWS-Edisto	13000	5770	125±17	210±57	754±26	1070±71	2620±131
SWS-Stroubles	12000	6190	99±30	136±17	1410±238	1860±301	1100±162
AQU-Columbia Deep	1185	724	17±1.8	20±1.1	30±0.3	49±4.2	124±1.6
AQU-Columbia Shallow	187	149	5±0.6	23±0.2	19±3.8	26±0.8	54±1.0

The quantity of TOC_s and soft carbon in a sediment sample affected the quantity of TOC_{aq} removed by the extractions. The two surface-water sediments with similar TOC_s and soft carbon contents released similar quantities of TOC_{aq} when TOC_{aq} values between the two sediments are compared for a specific extracting solution. Deep AQU-Columbia sediment that contained a factor of ten less TOC_s produced a factor of ten less TOC_{aq} across the different extracting solutions. Although the deep AQU-Columbia sediment contained 10× more TOC_s than the shallow AQU-Columbia sediment, the extracted TOC_{aq} values were similar for shallow and deep AQU-Columbia sediment. The high soft carbon content of shallow AQU-Columbia sediment compared to the soft carbon content of deep AQU-Columbia sediment provided insight into the

relationship between soft carbon content and the extractability of sediment associated organic carbon. These results indicate that high soft carbon content corresponds to increased TOC_{aq}. Based on the qualitative description of soft carbon (Weber et al. 2001), the direct relationship between soft carbon content and extractable TOC_{aq} is expected and indicated that extractable TOC_{aq} is a subset of soft carbon.

The average extraction efficiencies, defined as $TOC_{aq}/TOC_s \times 100\%$, are shown in Figure 3-1 for each sediment sample and extracting solution. Overall, the extraction efficiencies were higher for the low-carbon sediment (shallow AQU-Columbia) in comparison to the other sediments with some exceptions (SWS-Stroubles sediment – 0.1% and 0.5% pyrophosphate extractions). As state previously, the high quantities of extractable TOCaq in the shallow AQU-Columbia sediment is related to its soft carbon content. Although the SWS-Edisto and SWS-Stroubles sediments followed similar trends in the efficiency of the treatments, the SWS-Stroubles sediment differed from the SWS-Edisto sediment in several aspects. The 0.5% and 1.0% pyrophosphate extractions of SWS-Stroubles sediment were twice as effective in removing organic carbon in comparison to the SWS-Edisto sediment. However, the base extraction for the SWS-Edisto sediment removed twice the quantity of organic carbon compared with the SWS-Stroubles sediment. The different pools of organic carbon comprising the SWS-Edisto and SWS-Stroubles sediments are most likely responsible for the differences in organic carbon extractability. For example, the high levels of humic substances found in the Edisto River could explain its high quantity of base extractable carbon.

The increase in the extraction efficiency base extractions relative to pyrophosphate and Nanopure™ water suggests that the quantity of extracted carbon increases as the solution pH increases. However, the extraction mechanisms for pyrophosphate and NaOH are presumed to be different. For carbon extraction using sodium pyrophosphate, Stevenson (1994) proposed that the main extraction mechanism is complexation of persulfate with polyvalent cations resulting in organic matter solubilization. In contrast, base solutions de-protonate acidic functional groups to generate soluble forms and disrupt the bonds between the organic carbon and the inorganic sediment. For the shallow AQU-Columbia sediment, the small range of TOC_{aq} (20-26 mg/kg) for the three pyrophosphate extractions supports the contention that multiple extraction mechanisms are removing the organic carbon. If pH were the sole mechanism, the TOC_{aq} would have increased substantially over the three pyrophosphate extractions in the shallow AQU-

Columbia sediment. Furthermore, the base extraction for SWS-Stroubles sediment exhibited a two-fold decrease in TOC_{aq} compared with the 1.0% pyrophosphate extraction which also illustrates that pH changes can not be the only extraction mechanism.

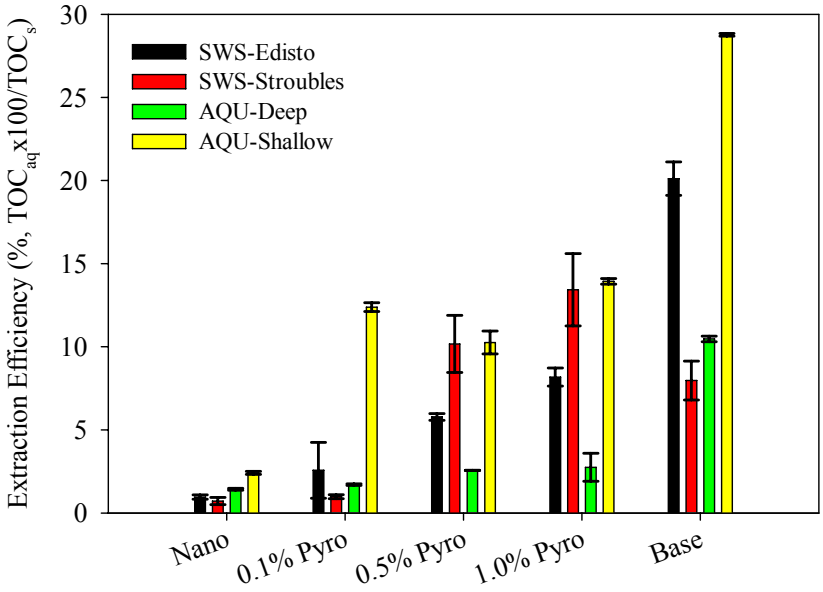

Figure 3-1. Extraction efficiencies (% of TOC_s removed during a single extraction) using Nanopure™ water, three concentrations of pyrophosphate, and base solutions for sediments of decreasing carbon (SWS-Edisto, SWS-Stroubles, Deep AQU-Columbia, and Shallow AQU-Columbia, respectively).

Bioassay Experiments

Bioassays were carried out using the organic carbon extracts as the sole carbon source for a mixed culture of soil microorganisms. In the bioassay experiments, SWS-Stroubles was selected as the test sediment because of unlimited availability to SWS-Stroubles sediment while quantity and accessibility to the AQU-Columbia and SWS-Edisto sediment sites were limited. The extent of carbon utilization was determined by the decrease of TOC_{aq} and production of headspace CO_2 relative to control bioassays over a relatively brief duration of time (<45 days). As shown in Figure 3-2a, the Nanopure™ water and 0.1% pyrophosphate extract bioassays followed a similar trend with 39% and 28% of the extracted carbon utilized within 43 days, respectively. The bioassays using the 0.5% and 1.0% pyrophosphate extractions also exhibited similar results with a 22% TOC_{aq} loss over 43 days in both cases. For the base extract, the bioassays resulted in a 40% TOC_{aq} loss over 43 days while the sterile control bioassays exhibited negligible loss in

TOC_{aq}. The increase in headspace CO_2 concentrations shown in Figure 3-2b supports the observed trend of TOC_{aq} loss over time in Figure 3-2a. The highest levels of CO_2 corresponded to the largest TOC_{aq} loss while the lowest levels of CO_2 corresponded to the lowest TOC_{aq} loss. Control data in Figure 3-2a and Figure 3-2b show that the loss of TOC_{aq} and production of CO_2 are the results of biotic processes. The combination of TOC_{aq} loss and CO_2 evolution shows that the carbon extracts contain readily biodegradable organic carbon that can potentially serve as electron donors for reductive dehalogenation.

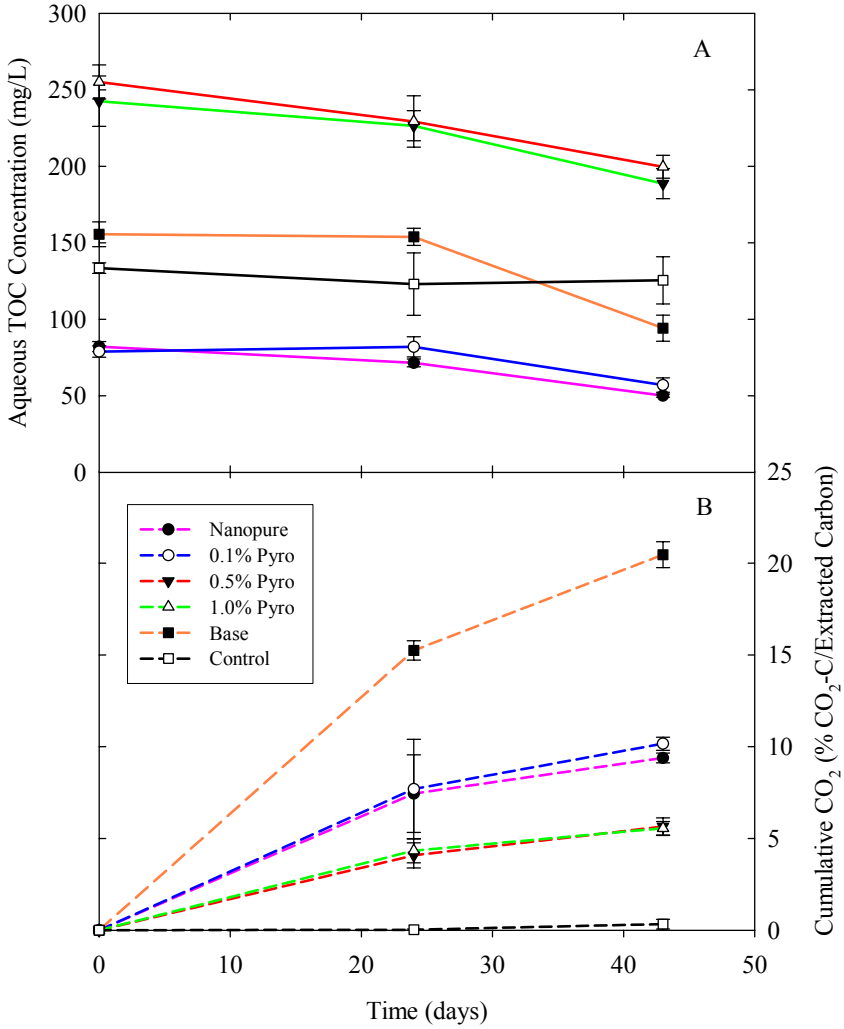

Figure 3-2. A) Decrease in TOC_{aq} concentration (solid lines) over time and B) increase in cumulative CO_2 (dotted lines) over time for Nanopure™ water, 0.1% pyrophosphate, 0.5% pyrophosphate, 1.0% pyrophosphate, base, and control base extractions of SWS-Stroubles sediment.

In Figure 3-3, the percentage of TOC_{aq} (relative to the sediment TOC_s) utilized during the bioassay experiments is compared with extraction efficiency for each sample. For the base extractions, a higher percentage of extracted carbon was biodegraded compared to the other extracts. Because the base extraction targets humics, it might be expected that the complex humic macromolecules would not degrade easily. However, the bioassay data show the opposite trend. It is assumed that the base extraction is hydrolyzing the complex macromolecules to produce a more readily usable carbon and energy source. For the 0.5% and 1.0% pyrophosphate extractions, less than 3% of the TOC_s was degraded even though the extractions removed more than 11% of the TOC_s. Although the SWS-Stroubles base extractions removed less carbon than the 0.5% and 1.0% pyrophosphate extractions, the base extractions contained more biodegradable organic carbon than the pyrophosphate extractions. For the less-aggressive extractants, the 0.1% pyrophosphate extractions removed 58 mg/kg more organic carbon than the Nanopure™ water extractions. In the bioassays, 8 mg/kg more of the organic carbon in the 0.1% pyrophosphate extracts was utilized than in the Nanopure™ water extracts. These results combined with the differences observed in the sediment extraction efficiencies suggest that the extracting solution removed different types of organic carbon that are subject to different degrees of biodegradation.

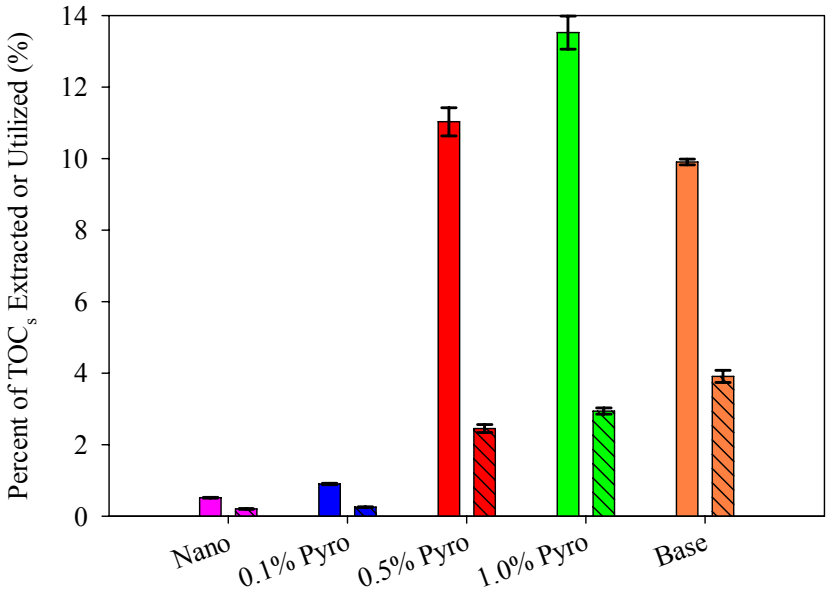

Figure 3-3. Percent of extracted TOC_{aq} relative to the TOC_s (plain bars) and percent of extracted TOC_{aq} utilized in the bioassays relative to the TOC_s (hatched bars) for Nanopure™ water, 0.1% pyrophosphate, 0.5% pyrophosphate, 1.0% pyrophosphate, and base extractions of SWS-Stroubles sediment.

The results for carbon extraction using the high-carbon sediments are similar to other experimental studies of carbon in soils. Using agricultural and forest soils and Nanopure™ water as an extracting solution, Boyer and Groffman (1996) and Trulleyova and Rulik (2004) reported 0.5-4% of the sediment TOC_s was extracted. In Figure 3-1, the Nanopure™ water extractions of surface water sediments and aquifer sediments removed 1.6-2.2% of the TOC_s. Therefore, the quantity of organic carbon extracted by Nanopure™ water in this study is within the same range as agricultural and forest soils. With respect to extracted carbon biodegradability, reported values from soil studies range from 10-40% of the extracted carbon biodegraded over a 45-60 day time frame (Schnabel et al. 2002; Gregorich et al. 2003). In this investigation, slightly higher biodegradability of extracted organic carbon was seen, with 30-60% of the TOC_{aq} biodegrading.

Multiple Extractions

Previous studies with forest and agricultural soil (Marschner and Kalbitz 2003) utilized single extractions that may not adequately exhaust the biodegradable organic carbon associated with the soil. Based on the low percentage of carbon extracted relative to the measure of soft carbon in this investigation, a two-step extraction sequence was run to test the remaining supply of sediment associated organic carbon on the SWS-Stroubles sediment. As Figure 3-5 shows, the sequential base extractions removed similar quantities of carbon regardless of the initial extractant or the amount of carbon extracted in the initial extraction. The average quantity of organic carbon removed in the second base extraction (Figure 3-5, 1240±150 mgC/kg) is similar to the average quantity of organic carbon extracted in the single extractions (Figure 3-1, 1100±160 mgC/kg). The results showed that more than one extraction is necessary to exhaust the supply sediment associated organic carbon, and the results indicated that different pools of organic carbon are extracted by different extracting solutions.

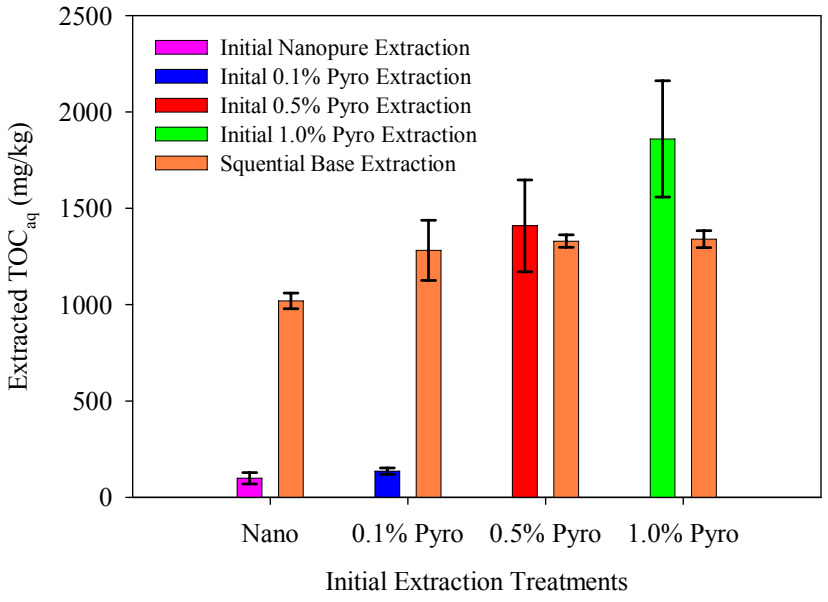

Figure 3-4. Quantity of extracted TOC_{aq} (mg/kg) for a two-step extraction sequence on SWS-Stroubles sediment where the standard deviations of triplicate extractions are shown with error bars. The initial extractions were run in parallel and used Nanopure water, 0.1% pyrophosphate, 0.5% pyrophosphate, or 1.0% pyrophosphate as an extracting solution. The second extraction run in sequence used 0.5 N sodium hydroxide (base) as an extracting solution in all the samples.

Based on the results of the two-step extraction sequence, a multiple extraction experiment was undertaken to investigate the ability of repetitive extractions to exhaust the supply of extractable and biodegradable carbon. The base and 0.1% pyrophosphate extracting solutions were selected for further investigation. The base extracting solution was selected because of its ability to remove a different pool of carbon compared to the other extracting solutions, and the 0.1% pyrophosphate extracting solution was selected due to its biodegradability relative to the other extracting solutions. Multiple 24-hour extractions using either 0.1% pyrophosphate or base were run on SWS-Stroubles sediment for eight repetitive extractions and on AQU-Columbia (shallow and deep) sediment for twenty repetitive extractions. Alternating 0.1% pyrophosphate/base extractions, where 0.1% pyrophosphate was used in the odd numbered extractions and base was used in the even numbered extractions, were also run for a total of eight extractions on AQU-Columbia and SWS-Stroubles sediment.

Figure 3-5 shows the cumulative extraction efficiencies (based on % of TOC_s) over the eight repetitive and eight alternating extractions using the SWS-Stroubles sediment. The initial base and 0.1% pyrophosphate extractions removed similar quantities of carbon as previously observed for these extractions in the single extraction experiments (Table 3-1). Subsequent base and 0.1% pyrophosphate extractions removed carbon linearly at an incremental increase of 345±33 mg/kg carbon per extraction with the exception of extraction 5 (E5). Over the eight extractions, the alternating 0.1% pyrophosphate/base extractions removed more organic carbon than the 0.1% pyrophosphate extractions but less than the base extractions. After E3 in the alternating extractions, the extracted carbon increased by 7.8%. This jump in extracted organic carbon indicates that exposure to base followed by 0.l% pyrophosphate improved the ability to extract carbon in comparison to repetitive 0.1% pyrophosphate extractions. After E2, the alternating 0.1% pyrophosphate/base extractions also extracted carbon with a similar incremental mass to the repetitive 0.1% pyrophosphate and base extractions.

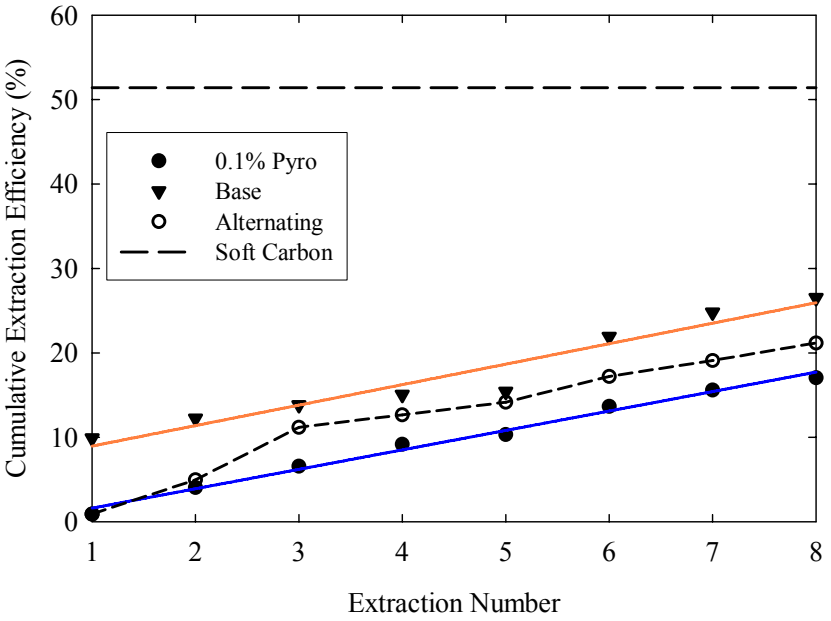

Figure 3-5. Cumulative extraction efficiencies from repetitive (solid line) and alternating (dotted lines) extractions of SWS-Stroubles sediment. Repetitive extractions used the same extractant over 8 extractions, and the 0.1% pyro alternating extraction alternated between 0.1% pyro (E1, E3, E5, E7) and base (E2, E4, E6, E8) extractants.

The repetitive extraction results for the AQU-Columbia sediments are shown in Figure 3-6. In shallow AQU-Columbia extractions (upper plot), the cumulative extraction efficiencies displayed similar trends during the first eight extractions to the SWS-Stroubles extractions. The alternating 0.1% pyrophosphate/base extractions removed more organic carbon than the 0.1% pyrophosphate extractions but less than the base extractions. Deep AQU-Columbia alternating extractions, however, exceeded the repetitive base extractions on E3 as a result of a jump in extracted carbon after exposure to base. After E3, deep AQU-Columbia alternating extractions (lower plot) exhibited a similar trend in carbon removal to the repetitive 0.1% pyrophosphate and base extractions. Overall, the expansion to twenty extractions for the shallow and deep AQU-Columbia sediments revealed an asymptotic approach to maximum values of extractable organic carbon.

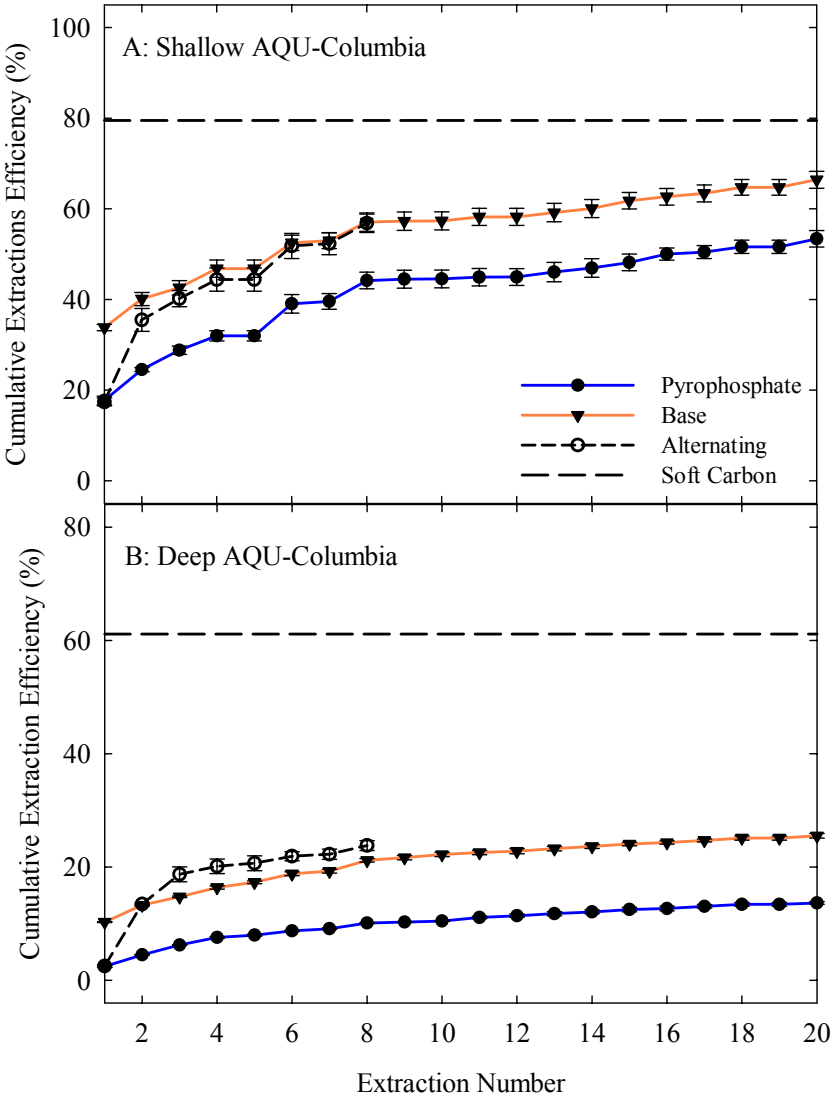

Figure 3-6. Cumulative extraction efficiency from repetitive (solid line) and alternating (dotted lines) extractions using AQU-Columbia sediment from shallow (A) and deep (B) depths. Repetitive extractions used the same extractant over 8 extractions, and the 0.1% pyro alternating extraction alternated between 0.1% pyro (E1, E3, E5, E7) and base (E2, E4, E6, E8) extractants.

Although the repetitive eight extractions using the SWS-Stroubles sediment increased in extractable organic carbon in a linear fashion, the extractions on shallow and deep AQU-Columbia sediments generated TOC_{aq} data that fit a rectangular hyperbola pattern (see Table 3-2 for regression parameters). Shallow AQU-Columbia data showed asymptotic maximums for the 0.1% pyrophosphate and base repetitive extractions at 59.2% and 67.3% of the TOC_s, respectively. Relative to soft carbon content (79.5% of the TOC_s), multiple extractions on

shallow AQU-Columbia sediment removed organic carbon within 80% of the soft carbon content. For deep AQU-Columbia multiple extractions, the asymptotes for the 0.1% pyrophosphate and base repetitive extractions were 17.3% and 27.9% of TOC_s, respectively. The multiple extractions on the deep AQU-Columbia sediment removed less than 50% of its soft carbon content (61.1% of TOC_s).

Table 3-2. Linear and rectangular hyperbola regression fit data for multiple extractions using either 0.1% pyrophosphate or base solution on SWS-Stroubles sediment regression and shallow and deep AQU-Columbia sediments, respectively.

Sediment	Extraction	Fit Equation	Parameters		p value	R^2
SWS-Stroubles	Pyrophosphate	y=mx+b	m	2.30	<0.0001	0.9978
			b	-0.69	0.1878	
	Base	y=mx+b	m	2.42	0.0023	0.9939
			b	6.54	<0.0001	
AQU-Columbia Shallow	Pyrophosphate	y=(ax)/(b+x)	a	59.17	<0.0001	0.9984
			b	3.92	<0.0001	
	Base	y=(ax)/(b+x)	a	67.31	<0.0001	0.9979
			b	1.50	<0.0001	
AQU-Columbia Deep	Pyrophosphate	y=(ax)/(b+x)	a	17.32	<0.0001	0.9993
			b	5.84	<0.0001	
	Base	y=(ax)/(b+x)	a	27.97	<0.0001	0.9986
			b	2.59	<0.0001	

Bioassays on Multiple Extractions

SWS-Stroubles sediment bioassays were constructed using extracts from E1, E3, and E8 from each extracting solution to evaluate the relative extent to which extracted organic carbon biodegrades as carbon is removed from sediment following repetitive extractions. Figure 3-7 shows the maximum TOC_{aq} utilization (defined as $\Delta TOC_{aq} \times 100\%/TOC_s$) which was lost over 45 days for each of the sets of bioassays. The repetitive 0.1% pyrophosphate and alternating 0.1% pyrophosphate/base bioassays exhibited a similar carbon utilization pattern. The alternating 0.1% pyrophosphate/base bioassays, however, contained more biodegradable organic carbon in E3. This substantial increase in biodegradable organic carbon in the alternating bioassays was a

result of the increase in quantity of extractable organic carbon in E3. The percent of carbon utilized in alternating E3 bioassays was similar to the percent of carbon utilized in the base E1 bioassays. Because the increase in extracted organic carbon in E3 is attributed to the use of base extracting solution, the similar percentages in TOC utilization indicate that the base extraction removed the same type of carbon regardless of extraction order. By the eighth extraction, the bioassays showed the lowest levels of biodegradability. The results from E8 indicated that the majority of biodegradable organic carbon had already been extracted from the sediment. The base extraction bioassays indicated that the biodegradability of carbon was highest in E1 and decreased over the course of eight extractions. With the traditional role of base extractions removing a large quantity of organic carbon from soils in an aggressive manner (Stevenson 1994), these base extractions of sediments not only exhibited high carbon removal efficiencies but also produced the largest quantity of biodegradable organic carbon. However, it is possible that the nature of the extracted organic carbon was altered during the base extractions due to saponifaction (Stevenson 1994). As a result, these alterations may cause extracted carbon to be more biodegradable, especially compared to solid-associated carbon. Overall, bioassay experiments demonstrated that base extractions are biodegradable and that alternating extractions are most effective for removing the biodegradable organic carbon from sediment.

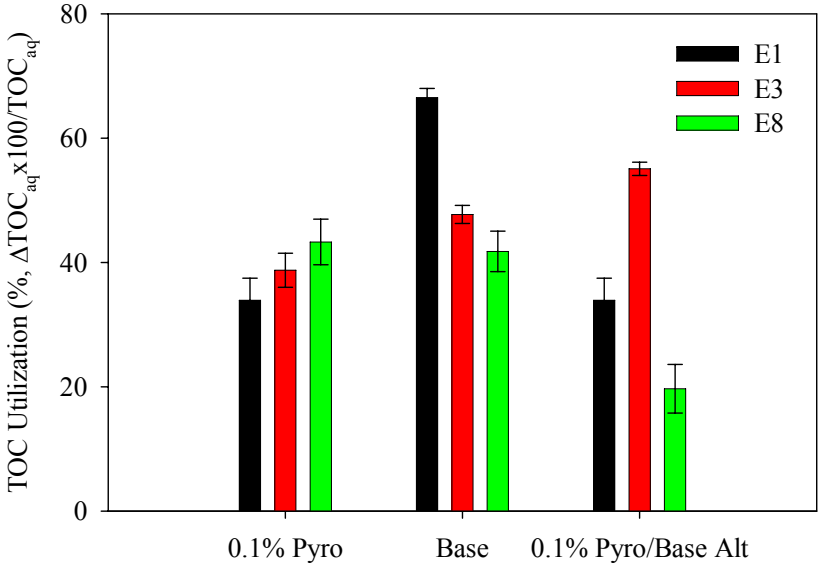

Figure 3-7. TOC_{aq} utilization efficiency (%, defined as $\Delta TOC_{aq} \times 100\%/TOC_s$) over 45 days for extraction 1 (E1), E3, and E8 of the continuous extractions using SWS-Stroubles sediment.

Proposed PBOC Method

Based on the results of the repetitive extractions and their biodegradability, a five-step extraction method is proposed to quantify the potentially bioavailable organic carbon (PBOC) associated with the aquifer sediment. The proposed PBOC extraction method consists of three sequential extractions with 0.1% pyrophosphate followed by one base extraction with a final 0.1% pyrophosphate extraction. The combination of the first three 0.1% pyrophosphate extractions, known as the pyrophosphate extraction, represents the loosely-extractable organic carbon associated with the sediment. Similarly, the combination of final two extractions, known as the alkali extraction, represents the relatively more strongly-associated organic carbon associated with the sediment. Sequential base extraction followed by 0.1% pyrophosphate extraction was selected because the combination of base followed by 0.1% pyrophosphate increased the removal efficiency of extractable biodegradable organic carbon from the sediment. The number of extractions was limited to five based on the continuous extractions results where extractable carbon decreased with each extraction. Overall, the series of five extractions will utilize the mild 0.1% pyrophosphate extractions and aggressive base extraction to target short term (labile) and long term sediment associated organic carbon, respectively.

Conclusions

Through the examination of single and multiple extractions and bioassay experiments using surface water and aquifer sediments, a better understanding of biodegradable organic carbon associated with sediment was gained. The results of extraction experiments showed that 1-38% of organic carbon associated with surface water and aquifer sediments can be extracted with a single extraction depending on the extracting solution. Further evaluation of the extracted organic carbon by bioassays demonstrated that 30-60% of the extracted organic carbon can be utilized over 45 days by soil microorganisms. Organic carbon removed by multiple extractions asymptotically approached a maximum value of extractable carbon. The biodegradability of the extracted carbon decreased with little to no biodegradable organic carbon after E8. However, one concern with the extraction followed by bioassay experimental sequence is the issue of in-situ organic carbon bioavailability when addressing sediment in an aquifer. The environmental conditions and release mechanisms by which the organic carbon associated with sediment becomes bioavailable are in need of investigation. Nevertheless, the extractions and bioassay

experiments provide the basis for developing an operational method to quantify the potentially bioavailable organic carbon in aquifer sediment.

Although the extracted organic carbon was not coupled directly with reductive dechlorination, the results of this paper are directed towards analyzing natural organic carbon associated with aquifer sediment and linking the organic carbon to sustainability of reductive dechlorination at chloroethene-contaminated sites. The ability of organic carbon to serve as the sole carbon source under oxic conditions suggests the potential of the organic carbon to ferment and generate hydrogen under anoxic environments. However, anoxic experiments where extracted organic carbon supports reductive dechlorination are needed. Furthermore, the acknowledgement of this potentially bioavailable organic carbon pool in aquifers is useful in discussion and prediction of reductive dechlorination sustainability.

References

Ball, W. P., C. H. Buehler, T. C. Harmon, D. M. Mackay and P. V. Roberts (1990). "Characterization of Sandy Aquifer Material at the Grain Scale." Journal of Contaminant Hydrology **5**(3): 253-295.

Boyer, J. N. and P. M. Groffman (1996). "Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles." Soil Biology & Biochemistry **28**(6): 783-790.

Bradley, P. M. (2000). "Microbial degradation of chloroethenes in groundwater systems." Hydrogeology Journal **8**(1): 104-111.

Chapelle, F. H., J. T. Novak, J. Parker, B. B. Campbell and M. A. Widdowson (2006). A Framework for Assessing the Sustainability of Monitored Natural Attenuation USGS Circular Series C-1303., United States Geological Survey.

Cuypers, C., T. Grotenhuis, J. Joziasse and W. Rulkens (2000). "Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments." Environmental Science & Technology **34**(10): 2057-2063.

Escobar, I. C. and A. A. Randall (2001). "Assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC): Complementary measurements." Water Research **35**(18): 4444-4454.

Gregorich, E. G., M. H. Beare, U. Stoklas and P. St-Georges (2003). "Biodegradability of soluble organic matter in maize-cropped soils." Geoderma **113**(3-4): 237-252.

Hawthorne, S. B., C. B. Grabanski, E. Martin and D. J. Miller (2000). "Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix." Journal of Chromatography A **892**(1-2): 421-433.

Leboeuf, E. J. and W. J. Weber (2000). "Macromolecular characteristics of natural organic matter. 2. Sorption and desorption behavior." Environmental Science & Technology **34**(17): 3632-3640.

Lucena, F., J. Frias and F. Ribas (1991). "A New Dynamic Approach to the Determination of Biodegradable Dissolved Organic-Carbon in Water." Environmental Technology **12**(4): 343-347.

Luthy, R. G., G. R. Aiken, M. L. Brusseau, S. D. Cunningham, P. M. Gschwend, J. J. Pignatello, M. Reinhard, S. J. Traina, W. J. Weber and J. C. Westall (1997). "Sequestration of hydrophobic organic contaminants by geosorbents." Environmental Science & Technology **31**(12): 3341-3347.

Marschner, B. and K. Kalbitz (2003). "Controls of bioavailability and biodegradability of dissolved organic matter in soils." Geoderma **113**(3-4): 211-235.

Pignatello, J. J. and B. S. Xing (1996). "Mechanisms of slow sorption of organic chemicals to natural particles." Environmental Science & Technology **30**(1): 1-11.

Schnabel, R. R., C. J. Dell and J. A. Shaffer (2002). "Filter, inoculum and time effects on measurements of biodegradable water soluble organic carbon in soil." Soil Biology & Biochemistry **34**(5): 737-739.

Schwarzenbach, R. P., P. M. Gschwend and D. M. Imboden (2003). Environmental Organic Chemistry. Hoboken, NJ, Wiley-Interscience.

Stevenson, F. J. (1994). Humus Chemistry: Genesis, Composition, Reactions. NY, NY., John Wiley and Sons, Inc. .

Trulleyova, S. and M. Rulik (2004). "Determination of biodegradable dissolved organic carbon in waters: comparison of batch methods." Science of the Total Environment **332**(1-3): 253-260.

Volk, C. J. and M. W. LeChevallier (2000). "Assessing biodegradable organic matter." Journal American Water Works Association **92**(5): 64-76.

Weber, W. J., E. J. Leboeuf, T. M. Young and W. L. Huang (2001). "Contaminant interactions with geosorbent organic matter: Insights drawn from polymer sciences." Water Research **35**(4): 853-868.

Weber, W. J., P. M. McGinley and L. E. Katz (1992). "A Distributed Reactivity Model for Sorption by Soils and Sediments. 1. Conceptual Basis and Equilibrium Assessments." Environmental Science & Technology **26**(10): 1955-1962.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, E. K. Gorden, J. T. Wilson, B. H. Wilson, D. H. Kambell, P. Haas, R. N. Miller, J. E. Hansen and F. H. Chapelle (1998). Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. Washington, DC, U.S. EPA.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, J. T. Wilson, D. H. Kambell, J. E. Hansen and P. Haas (1996). Overview of the technical protocol for natural attenuation of chlorinated aliphatic hydrocarbon in groundwater under development of the U.S. Air Force Center for Environmental Excellence. Proceedings of the Syposium on Natural Attenuation of Chlorinated Solvents, Washington, DC, U.S. EPA.

Xing, B. S. and J. J. Pignatello (1997). "Dual-mode sorption of low-polarity compounds in glassy poly(vinyl chloride) and soil organic matter." Environmental Science & Technology **31**(3): 792-799.

Yang, Y., D. Ratte, B. F. Smets, J. J. Pignatello and D. Grasso (2001). "Mobilization of soil organic matter by complexing agents and implications for polycyclic aromatic hydrocarbon desorption." Chemosphere **43**(8): 1013-1021.

Chapter 4. Anaerobic utilization of potentially bioavailable organic carbon from aquifer sediment to support reductive dechlorination

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Abstract

In this study, the relationship between potentially bioavailable organic carbon (PBOC) extracted from aquifer sediment samples and reductive dechlorination of chloroethenes was investigated. Using extractable organic carbon from the PBOC method as the sole electron donor, enrichment culture experiments populated with dechlorinating and fermenting microorganisms exhibited reductive dechlorination of tetrachloroethene through vinyl chloride in laboratory microcosms. In anaerobic bioassays constructed using extractable organic carbon from the PBOC method and fermenting microorganisms, hydrogen was produced at levels consistent with those necessary to drive reductive dechlorination (> 1 nM). The results of the reductive dechlorination enrichment culture and anaerobic bioassay experiments show that PBOC extracts can support reductive dechlorination. Anaerobic and aerobic bioassays investigated the extent to which extracted PBOC was biodegraded by monitoring organic carbon utilization. Over 45 days, aerobic bioassays exhibited a decrease in aqueous organic carbon in all bioassays while anaerobic bioassays only exhibited a decrease in aqueous organic carbon for 50% of the bioassays. The results indicated that anaerobic bioassays may require a longer incubation time to observe organic carbon utilization, and the percent of carbon utilized was similar between the aerobic and anaerobic bioassays (24-32%) for the anaerobic bioassays exhibiting a change in aqueous organic carbon.

Introduction

The widespread use of the chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) in industrial processes has resulted in PCE and TCE being ubiquitous contaminants in sediment and aquifer environments (Wiedemeier et al. 1998). Although originally regarded as recalcitrant, chlorinated solvents are now known to be biodegradable via multiple pathways

(Vogel and McCarty 1985; DiStefano et al. 1991; Bradley and Chapelle 1996; Roberts et al. 1996; Butler and Hayes 1999). One widely-observed degradation pathway in anaerobic ground-water systems is reductive dechlorination, in which chloroethenes serve as electron acceptors for *Dehalococchoides* sp. and other dechlorinating bacteria. Microorganisms sequentially replace chlorine with hydrogen to produce daughter products such as cis-1,2-dichloroethene (*cis*DCE), vinyl chloride (VC), ethene, and ethane (Debruin et al. 1992; Mohn and Tiedje 1992; Maymo-Gatell et al. 1997). For dehalogenation, research has shown the predominant electron donor is molecular H_2 (Holliger and Schumacher 1994; Maymo-Gatell et al. 1995; Smatlak et al. 1996; Yang and McCarty 1998) although acetate has also been found to be as an effective electron donor for reductive dechlorination by some dechlorinating organisms (Krumholz et al. 1996; He and Sanford 2002). The production of acetate and H_2 is attributed to the subsurface fermenting community that utilizes complex organic carbon (McCarty and Smith 1986; Fennell et al. 1997).

At chlorinated-solvent sites, the source of the reducing compounds, typically organic carbon, in an aquifer can originate from either anthropogenic inputs (Type I site) or indigenous sources (Type II site) (Wiedemeier et al. 1996). This study focuses on Type II sites where natural organic carbon (NOC) associated with the aquifer sediment and ground-water matrix provides fermentable organics. Biodegradable NOC in a ground-water system can originate in the water column as recharge passes through the vadose zone and as ground water is conveyed through the aquifer and confining units (Chapelle et al. 2006). At Type II sites, assuming that the supply of electron donor is derived from the fermentation of NOC, the ability to measure NOC is needed to assess the potential for the long-term sustainability of reductive dechlorination (Chapelle et al. 2006).

In the previous chapter, a measure of bioavailable NOC in aquifer sediment was investigated using liquid extraction techniques, and the extent to which the extracted carbon biodegrades was evaluated. Single extractions with different extracting solutions (Nanopure™ water, sodium hydroxide, and sodium pyrophosphate) showed that the range of extractable organic carbon associated with the sediment is 1-38% of the total organic carbon content of the sediment. Bioassay studies of the single extractions demonstrated that 30-60% of the organic carbon extracted can be utilized by a microbial consortium. The extractable carbon from repetitive extractions on aquifer sediment using either 0.1% pyrophosphate or base solutions asymptotically approached a maximum value. Alternating extracting solutions between 0.1%

pyrophosphate and sodium hydroxide increased the removal rate efficiency and showed that two pools of organic carbon could be extracted. The bioassays of alternating extractions showed that the extent to which the extracted carbon was biodegraded peaked at the third extraction and was negligible by the eighth extraction. The result of the investigation was a laboratory method to quantify organic carbon from the aquifer matrix in terms of potentially bioavailable organic carbon (PBOC).

Because the initial experiments were conducted under aerobic conditions and were not designed to demonstrate reductive dechlorination of chloroethenes, an experimental proof-of-concept step was required. It was hypothesized that the organic carbon extracted by the PBOC method contributes to the sustainability of reductive dechlorination by serving as source of electron donor. To address this hypothesis, experiments were conducted to assess the contribution of PBOC to reductive dechlorination in laboratory microcosms and bioassays. First, experiments using an enrichment culture populated with dechlorinating and fermenting microbes investigated the ability of the PBOC extracts to serve as the electron donor for reductive dechlorination. In addition, anaerobic bioassays were run to evaluate the production of hydrogen gas and volatile fatty acids (VFAs) through the utilization of the PBOC extractions under anaerobic conditions. By monitoring the utilization of PBOC extracts under anaerobic conditions for fermentation intermediates and H_2 production, the complex organic carbon fermentation pathway in an aquifer was investigated for the potential for the PBOC extracts to provide electron donor for reductive dechlorination. Carbon utilization results for aerobic and anaerobic bioassay experiments using aquifer sediment from the same sample locations and depths were also compared.

Materials and Methods

Site Background

The Naval Amphibious Base Little Creek (NABLC) Site 12 in Virginia Beach, VA, was home to a former laundry/dry cleaning facility. In the early 1970's the dry cleaning facility discharged waste containing PCE and other organic byproducts into a manhole that fed into a storm sewer catch drain. An estimated 200 gallons of PCE and 1100 gallons of soaps, sizing, and dyes were discharged into the storm sewer (CH2MHill 2000). Based on contaminant concentrations in ground-water and aquifer sediment samples and in-situ characterization using a

membrane interface probe, two source zones were identified. PCE, TCE, and *cis*DCE have been detected in the ground water in and down gradient of the source zones. VC observation in ground water is inconsistent throughout the site. Redox indicator data and contaminant concentrations in the ground water indicate that reductive dechlorination is occurring at the site and contributes to the natural attenuation of the chloroethenes at Site 12 (CH2MHill 2000).

Sediment Samples

Aquifer sediment was collected from two sampling sites in the Columbia Aquifer (AQU-Columbia) which is located in the Atlantic Coastal Plain of Virginia. The first sampling location, MLS10, was selected to provide aquifer sediment from a location upgradient of the contaminant source and plume. The second sampling location, MLS12, was identified during site characterization as a location with the highest site concentrations of PCE in ground water (> 10 mg/L). Both locations were sampled at depths of 8-10 ft and 20-22 ft below land surface (henceforth referred to as shallow and deep, respectively). Aquifer sediment samples were collected with a Geoprobe unit using sterile acetate liners. All samples were stored intact, sealed in nitrogen-filled bags, and kept at 4°C until analysis.

PBOC Extractions

PBOC extractions followed the method outlined in the previous chapter. Dried, sieved aquifer sediment was combined with an extracting solution using a solution:sediment ratio of 2:1. As operationally defined in the Chapter 3, the PBOC consists of a five step extraction process where three 24-hour extractions with 0.1% pyrophosphate are followed with a 24-hour 0.5 N NaOH extraction followed by an additional 24-hour 0.1% pyrophosphate extraction. The combination of the first three 0.1% pyrophosphate extractions was referred to as the pyrophosphate extract and represents the loosely-extractable organic carbon associated with the sediment. Similarly, the combination of the final two extractions was reported as the alkali extract and represents the more strongly-associated organic carbon associated with the sediment.

Enrichment Culture

Two enrichment cultures were developed under anaerobic conditions using sediment samples taken at MLS12 based on ground-water analyses that showed reductive dechlorination daughter products. One enrichment culture used MLS12 shallow sediment for the seed dechlorinating

culture. The ground-water contaminant concentrations at MLS12 shallow showed reductive dechlorination activity through VC and ethene production (Rectanus 2000). Therefore, the potential for complete reductive dechlorination existed in these enrichment cultures. The second enrichment culture was grown with deep sediment from MLS12. Because no VC has been observed in the ground water in the deep depths at MLS12, the enrichment cultures were not expected to produce VC.

Enrichment culture setup included 10 g sediment and 90 mL of minimal salts media, Wolin vitamins, and yeast extract. The constituents of the minimal salts media are listed in Chapter 3. The Wolin vitamin stock was based on Mattson (2004). Yeast extract (1%) was added to the enrichment cultures to provide a source of electron donors for the fermentative and dechlorinating microorganisms. After a two week equilibration period, PCE (4 μM) was added to the enrichment cultures. The aqueous PCE concentration and subsequent daughter product formation was monitored over time. PCE and its daughter products were quantified using a purge and trap method (Rectanus 2000). The enrichment culture was maintained by transferring 10 mL of culture into 90 mL of fresh minimal salts media, Wolin vitamins, yeast extract, and PCE approximately every two weeks (transfer was based on loss of aqueous PCE). Following the fourth transfer, the PBOC extract experiment was run. The PBOC experiment consisted of 10 mL of enrichment culture, 5 mL of minimal salts media (10× stock), 0.25 mL Wolin vitamins, and 50 mL of PBOC extracts from outside of the plume (MLS10). PCE (4 μM) was added to the PBOC enrichment culture experiments last to minimize loss. Aqueous PCE, TCE, *cis*DCE, and VC production was monitored over time using the purge and trap method.

PBOC Bioassays

Aerobic bioassays were constructed using the method described in the previous chapter. Anaerobic bioassay experiments were constructed in a similar manner to the aerobic bioassays with the exception that the headspace of the 40-mL glass vials for both the live and control bioassays was purged with sterile N_2 to remove O_2 and facilitate anaerobic microorganism growth. The inoculum used in both the aerobic and anaerobic bioassays consisted of a consortium populated with facultative soil microbes. The inoculum extraction and growth methods are described in the previous chapter. The anaerobic bioassays were incubated on orbital shakers in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) with a 95%

N_2 and 5% H_2 atmosphere. The anaerobic bioassays were sampled for CH_4 and CO_2 generation and TOC utilization in a similar manner to aerobic bioassays.

The quantity of carbon utilized in the bioassays was defined as the maximum decrease in TOC_{aq} concentration and statistically supported by the non-parametric Kruskal-Wallis one-way analysis of variance based on ranks. All statistical tests were run with the NCSS software package (Kaysville, Utah) using α=0.05. The statistically similarity between the medians from the triplicate TOC_{aq} values for each time point was evaluated. The statistically dissimilar medians were compared to the stabilization in incremental headspace CO_2 production to verify maximum decrease in aqueous carbon utilization.

Volatile fatty acid (VFA) production was documented over the course of the anaerobic experiment in order to assess fermentation of the extracted PBOC. After each sampling round, the headspace of the bioassays was flushed with oxygen for the aerobic bioassays and N_2 for the anaerobic bioassays. Separate hydrogen experiments were conducted to monitor the potential production of hydrogen gas from pyrophosphate and alkali PBOC extracts on MLS10 sediment from NABLC. Experiments were constructed with similar solution ratios to bioassays but incubated in 150 mL serum vials to allow for 5 mL headspace samples. Experiments were incubated in an anaerobic chamber with a N_2 environment with negligible H_2.

Analytical Methods

Headspace CO_2 and CH_4 concentrations in the bioassays were determined with a gas chromatograph using a thermal conductivity detector (TCD, Shimatzu GC-14A). TOC_{aq} content for extraction and aerobic bioassay samples was analyzed via wet persulfate oxidation (Techmar Dohrmann DC-80). Aqueous TOC for the anaerobic bioassays was analyzed with catalytic combustion (Shimadzu TOC-V CSN Total Organic Carbon Analyzer). The solid-phase organic carbon content (TOC_s) was determined by elemental analysis (ThermoFinnigan Flash EA 1112). Soft carbon measurements followed the method outlined in the previous chapter, using an approach that was adapted from Cuypers et al. (2000). VFA concentrations were measured following the method in Kelly (2006). Headspace hydrogen measurements in the anaerobic bioassays were measured using the method described in Chapelle et al. (1996).

Results and Discussion

To establish the relationship between PBOC extracts from aquifer sediment and reductive dechlorination at a chloroethene-contaminated site, this study consisted of three parts: First, an enrichment culture populated with dechlorinating and fermenting microbes was developed and used to investigate the extent to which PBOC extracts serve as an electron donor for reductive dechlorination. Then, anaerobic bioassays were conducted to determine if and how much hydrogen gas and volatile fatty acids (VFA) were evolved due to utilization of the PBOC extracts under anaerobic conditions. Third, aerobic and anaerobic bioassay experiments were run to evaluate the effect of redox condition on the utilization of the PBOC extracts as the sole carbon source.

Reductive Dechlorination Culture

Pyrophosphate and alkali PBOC extracts were fed as the sole electron donor to an enrichment culture containing fermentative and dechlorinating microorganisms to test the ability of the extracts to support reductive dechlorination. Both the shallow and deep PBOC extracts from MLS10 were tested as electron donors for reductive dechlorination. Figure 4-1 depicts the dechlorination activity in terms of aqueous chloroethene concentrations over time for the enrichment cultures amended with pyrophosphate PBOC, alkali PBOC, or yeast extract. The results of the enrichment culture experiment demonstrate that the PBOC extracts can serve as electron donors to support reductive dechlorination activity.

For the MLS10 shallow sediment, pyrophosphate extracts (Figure 4-1A) supported dechlorination of PCE through *cis*-1,2-DCE with low levels of TCE and VC (0.33 μM) production. For the alkali extracts (Figure 4-1B), the enrichment culture experiments showed that the extracted organic carbon supported dechlorination of PCE through VC. The alkali extract amended cultures also produced low levels of TCE similar to the pyrophosphate extract amended cultures. The positive control culture with yeast extract (Figure 4-1C) exhibited dechlorination through *cis*-1,2-DCE with trace levels of TCE and VC (0.37 μM) production. All three enrichment cultures produced VC within 45 days and generated limited TCE production within the first 10 days.

For the MLS10 deep sediment, the pyrophosphate extracts (Figure 4-1D) supported reductive dechlorination through *cis*-1,2-DCE as expected. However, low levels of VC (0.44 μM) were

also observed. Because no VC has been observed in the ground water in the deep levels at MLS12, the enrichment cultures were not expected to produce VC. The alkali PBOC extracts dechlorinated PCE through VC (Figure 4-1E). These results indicate not only the ability of the PBOC extracts to support reductive dechlorination but also the capability of the dechlorinating population at MLS12 deep to generate VC. For the positive control experiments (Figure 4-1F), PCE was dechlorinated through *cis*-1,2-DCE with trace levels of VC (0.37 μM) detected. As in the shallow enrichment cultures, the pyrophosphate extract and positive controls cultures produced trace levels of VC and the alkali extract amended culture converted the majority of the spiked PCE into VC. Interestingly, no TCE was observed in the deep enrichment cultures as was observed in the shallow enrichment cultures.

Chloroethene mass balance calculations based on the 1:1 stoichiometeric conversion in reductive dechlorination indicated a loss between 20-50% of amended PCE after 45 days for the live experiments. Similarly, killed controls showed loss of the contaminants between 40-50% with negligible daughter products formation. It is assumed contaminant loss was due to sorption to the septa and leakage through the septa after repetitive sampling. The killed controls substantiated the biotic conversion of PCE through VC in the live experiments and had similar mass loss due to experimental conditions.

A contaminant mass balance based on the 1:1 stoichiometeric conversion in reductive dechlorination indicated a loss between 20-50% of amended PCE after 45 days for the live experiments. Similarly, killed controls showed loss of the contaminants between 40-50% with negligible daughter products formation. It is assumed contaminant loss was due to sorption to the septa and leakage through the septa after repetitive sampling. The killed controls substantiated the biotic conversion of PCE through VC in the live experiments and had similar mass loss due to experimental conditions.

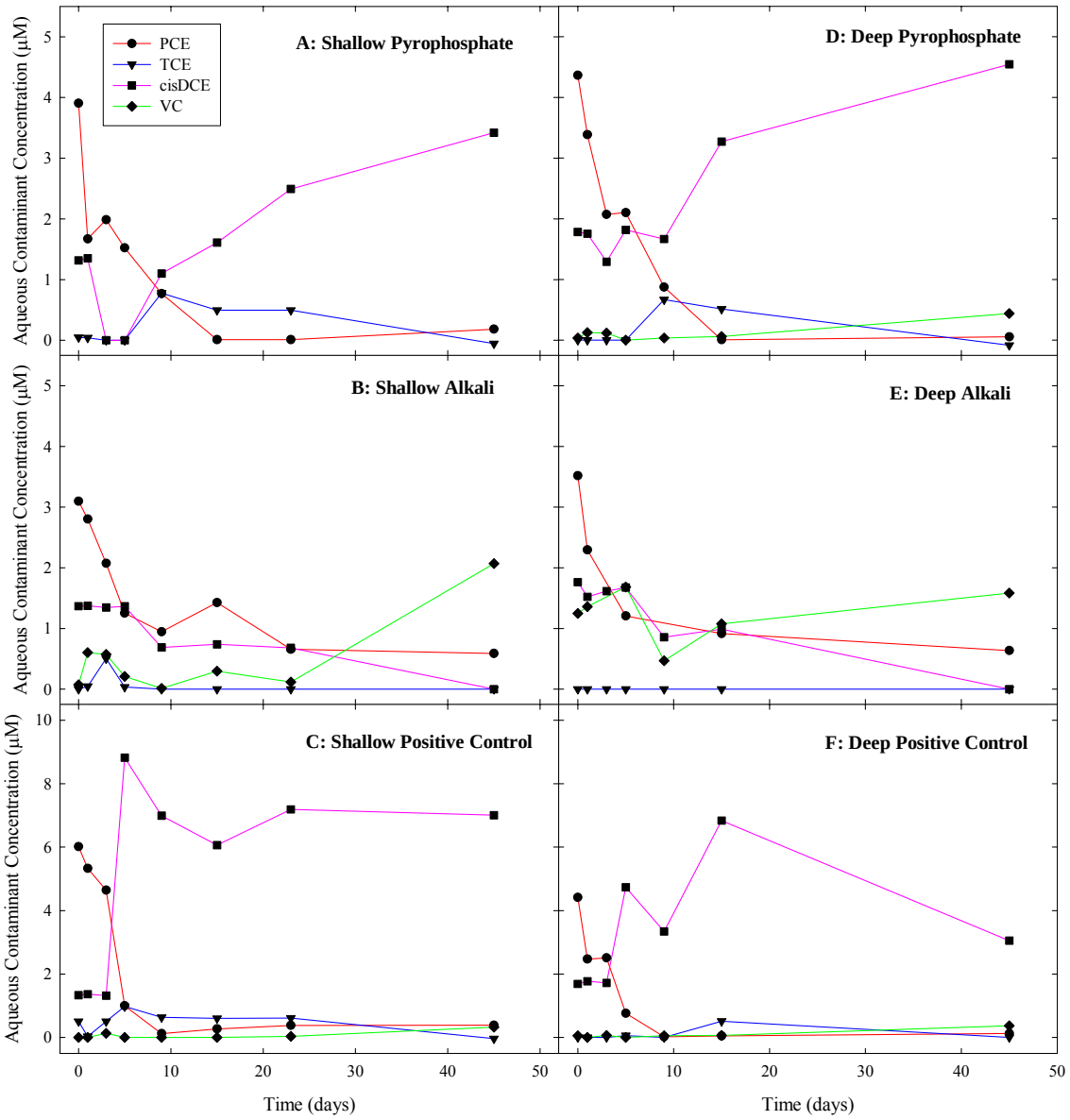

Figure 4-1. Summary of reductive dechlorination enrichment culture experiments where each subgraph illustrates the aqueous contaminant concentration over time for the following conditions: (A) MLS10 Shallow Pyrophosphate extract with the MLS12 Shallow enrichment culture, (B) MLS10 Shallow Alkali extract with the MLS 12 Shallow enrichment culture, (C) Positive control yeast extract with the MLS 12 Shallow enrichment culture, (D) MLS10 Deep Pyrophosphate extract with the MLS12 Deep enrichment culture, (E) MLS10 Deep Alkali extract with the MLS12 Deep enrichment culture, and (F) Positive control yeast extract with the MLS 12 Deep enrichment culture.

For both sets of enrichment cultures (shallow and deep), the alkali extracts supported dechlorination of PCE through VC. The shallow enrichment cultures converted 68% of the

initial PCE into VC. Similarly, 45% of the initial PCE was converted to VC in the deep enrichment cultures. However, in the pyrophosphate extract and positive control cultures, low VC concentrations were measured. The relative quantities of carbon in the PBOC extracts added to the enrichment cultures do not explain the differences in reductive dechlorination activity. The PBOC extracts from the shallow sediment contained similar quantities of carbon, 10.0 and 7.57 mg/L for the pyrophosphate and alkali extractions, respectively. While more organic carbon was released from the deep sediment (14.4 and 28.6 mg/L for pyrophosphate and alkali extracts, respectively) relative to the shallow sediment, the PBOC extracts from the shallow sediment produced more VC.

The first-order rates for PCE degradation are shown in Table 4-1. The PCE degradation in the enrichment cultures fit a first-order reaction with R^2 values greater than 0.94. The regression intercept at time = 0 corresponded well with the initial concentrations of PCE spiked into the cultures at the start of the experiment. The enrichment cultures exhibited dechlorination reaction rates ranging from 0.09 to 0.29 day^{-1}. These rate constants are in the same range as rates reported in Bouwer and McCarty (1983) and Parsons et al. (1984) for laboratory microcosms. In the shallow MLS12 enrichment cultures, the rate constants did not differ substantially between the positive controls and the pyrophosphate PBOC amended cultures. For the deep MLS12 enrichment cultures, the rate coefficient in the positive control was twice the rate coefficients for the PBOC extraction amended cultures. In contrast to the shallow cultures amended with pyrophosphate PBOC and yeast extract, the deep cultures indicated the change in organic carbon substrate impacted the rate of dechlorination. Furthermore, in the MLS12 shallow enrichment culture, the alkali extractions showed the lowest first order degradation rates and demonstrated the most VC production. Therefore, the type of organic carbon in the PBOC extraction may have contributed to the low reductive dechlorination rate and also enabled VC production.

Table 4-1. Summary of the PCE first-order degradation rates for the enrichment cultures amendment with PCE (4 uM).

Enrichment Culture	MLS10 PBOC Amendments	$C=C_0e^{-kt}$		R^2
		C_0 (μM)	k (1/day)	
MLS12 Shallow	Shallow Pyrophosphate	3.88	0.20	0.99
	Shallow Alkali	2.84	0.09	0.96
	Positive Yeast Extract	6.48	0.23	0.96
MLS12 Deep	Deep Pyrophosphate	4.22	0.18	0.99
	Deep Alkali	3.12	0.15	0.94
	Positive Yeast Extract	4.17	0.29	0.96

*The positive control was amended with 8 μM PCE.

Anaerobic Bioassays

Hydrogen Experiments

Experiments were conducted to monitor production of hydrogen from pyrophosphate and alkali PBOC extracts on MLS10 sediment from NABLC. Based on the enrichment culture experiments, it was shown that the PBOC extractions can support reductive dechlorination and should therefore produce H_2. As shown in Figure 4-2, both the pyrophosphate and alkali extracts exhibited hydrogen production. A conversion from headspace to aqueous concentrations using Henry's Law allowed the comparison of these hydrogen experiments to ground-water hydrogen concentrations. As discussed in Chapelle et al. (1996), ground-water hydrogen concentrations can be used to indicate the predominant microbial terminal electron accepting process (TEAP). The synergistic relationship between microbial communities is manifested through a minimum H_2 threshold that translates to ranges of H_2 representing the predominant microbial TEAP. For the shallow and deep MLS10 sediments, the pyrophosphate extracts produced aqueous hydrogen concentrations in the range of iron reduction (0.2-0.8 nM) during the first 14 days of the experiment and dropped to nitrate reducing hydrogen threshold levels (<0.1 nM) at day 28. After day 28, the pyrophosphate extract bioassays did not produce measurable hydrogen. For the alkali extracts from the shallow and deep MLS10 sediments, Figure 4-2 showed aqueous hydrogen levels between 7 and 50 nM which is in the range of methanogenesis. Although the quantity of TOC extracted in the shallow extractions was lower than the quantity of carbon extracted in the deep extractions (as reported in the enrichment culture section), the hydrogen production was dependent on the type of carbon in the extract, i.e. pyrophosphate or alkali

extraction. Therefore, the ability to produce H_2 was linked to the pool of carbon removed during the extraction not the total quantity of carbon. Most importantly, the hydrogen experiment showed that the PBOC extracts produce H_2 at levels able to drive reductive dechlorination.

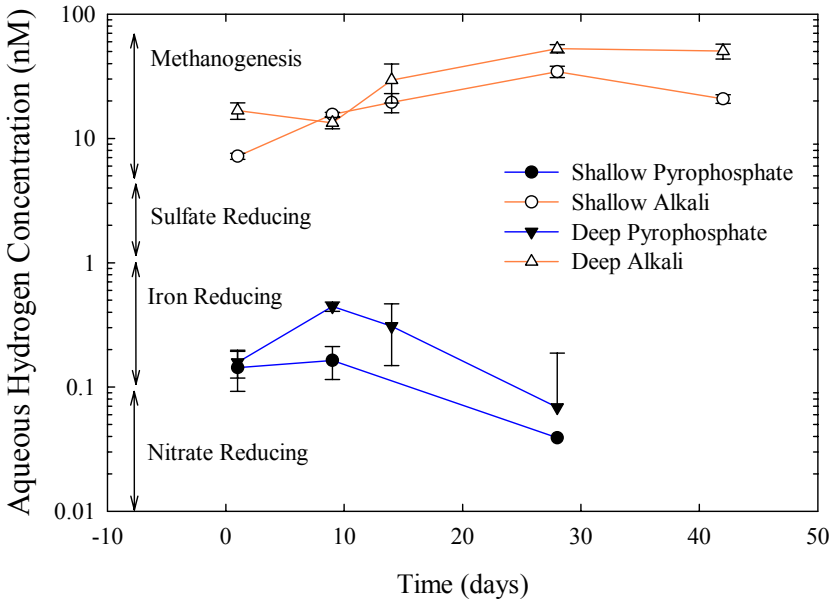

Figure 4-2. Aqueous hydrogen concentrations (nM) over time for pyrophosphate and alkali extracts on shallow (8-10 ft) and deep (20.5-22 ft) MLS10 sediment from NABLC.

The results of the hydrogen experiment support the results of the enrichment culture experiments. In the hydrogen experiments, the alkali extracts produced the highest levels of hydrogen. For the enrichment cultures, the alkali extracts also demonstrated complete reductive dechlorination of PCE through VC with approximately 50% of the PCE converted to VC. The partial dechlorination through *cis*-1,2-DCE shown in the pyrophosphate extracts from MLS10 shallow and deep enrichment cultures corresponds to the lower levels of hydrogen production observed in Figure 4-2. The lack of hydrogen production after 28 days indicates that the supply of appropriate electron donor may have been depleted in the enrichment culture experiments and resulted in *cis*-1,2-DCE accumulation. Based on the observed levels of hydrogen production seen in Figure 4-2, the results of the enrichment culture experiment indicated the ability to produce hydrogen can be linked to the extent of reductive dechlorination.

Volatile Fatty Acid Production

As stated previously, reducing compounds capable of generating volatile fatty acids (VFA) and H_2 are needed for active reductive dechlorination (Fennell et al. 1997). In Type II sites, it is assumed that natural organic carbon associated with the aquifer sediment goes through fermentation to produce intermediate VFAs before generating the end products, acetate and H_2. As production of H_2 and support of reductive dechlorination has already been demonstrated by the PBOC extractions, anaerobic bioassays were run to monitor for VFA production and assess TOC utilization (to be discussed in next section). In Table 4-2, a summary of the aqueous VFAs analyses is shown for the anaerobic MLS10 bioassays. Overall, low to no VFAs were observed during the anaerobic bioassay experiments. The pyrophosphate MLS10 shallow bioassays produced the most diverse quantity of VFAs while the alkali MLS10 deep bioassays produced the highest quantity of VFAs (as equivalents of acetic acid). It was assumed that the low levels and transitory nature of the VFAs restricted the ability to monitor their production. To evaluate this fermentation pathway, future work should consider interrupting the synergy between the hydrogen utilizing microorganisms and the fermenting organisms to allow for accumulation of VFAs.

Table 4-2. Summary of the aqueous Volatile Fatty Acid (VFA) analysis for the MLS10 anaerobic bioassays. Concentrations of VFAs are reported as equivalents of acetic acid (mg/L) based on the conversion of the VFA to acetate on a COD basis.

VFA	MLS10			
	Shallow		Deep	
	Pyrophosphate	Alkali	Pyrophosphate	Alkali
Acetic Acid	17.2	BDL	BDL	43.6
Propionic Acid	BDL	BDL	BDL	BDL
Isobutyric Acid	1.4	0.5	BDL	BDL
Butyric Acid	1.0	BDL	BDL	BDL
Isovaleric Acid	1.4	BDL	BDL	BDL
Caproic Acid	BDL	BDL	BDL	BDL
Isocaproic Acid	1.5	1.2	BDL	BDL
Hexanoic Acid	BDL	BDL	BDL	BDL
Heptanoic Acid	BDL	BDL	BDL	BDL
Total as equivalents of Acetic Acid (mg/L)	22.4	1.7	BDL	43.6

BDL: Below detection limit. Controls for MLS10 bioassays exhibited negligible VFA production except the pyrophosphate extraction bioassay from MLS10 Deep where 20 mg/L acetate was observed.

TOC Utilization

The combination of the enrichment culture and hydrogen experiments established that the PBOC extracts support anaerobic microorganism communities as the sole carbon substrate and electron donor. However, the biodegradability of the PBOC extracts had not been demonstrated under both anaerobic and aerobic conditions using TOC utilization as a metric. In the previous chapter, aerobic bioassays demonstrated that the biodegradability from single and multiple extractions was between 30-60% of the extracted TOC based on a decrease in TOC_{aq}. Therefore, anaerobic and aerobic bioassays were run using the pyrophosphate and alkali PBOC extracts as the carbon source.

The anaerobic and aerobic bioassays demonstrated that the PBOC extracts contained organic carbon capable of supporting either anaerobic or aerobic microorganisms. As shown in Table 4-3, the percent of carbon utilized in the aerobic and anaerobic bioassays was similar for the bioassays demonstrating PBOC extract biodegradability. The lack of TOC_{aq} loss in the shallow pyrophosphate and deep alkali extracts is not fully understood but may be linked to the production of VFAs (Table 4-2). While a decrease was not observed in the TOC_{aq} for the shallow pyrophosphate and deep alkali extract bioassays, VFA production was observed. The VFAs would have been included in the TOC_{aq} concentrations even though transformation of the PBOC extracts was occurring. The results in Table 4-3 confirm the use of aerobic bioassays in Chapter 3 that demonstrate the biodegradability of the PBOC extractions. Furthermore, the results indicated that anaerobic bioassays may require a longer incubation time to observe organic carbon utilization.

Table 4-3. Summary of percent carbon utilized by the aerobic and anaerobic bioassays for pyrophosphate and alkali extractions of MLS10 and MLS20 sediment samples.

Sample	Depth	Pyrophosphate Extractions		Alkali Extractions	
		Aerobic % Utilized	Anaerobic % Utilized	Aerobic % Utilized	Anaerobic % Utilized
MLS10	8-10 ft	12.2%	0*	23.6%	24.0%
MLS10	20.5-22 ft	32.1%	31.7%	53.7%	0*

* reflects no statistical degradation in aqueous TOC concentrations during the experiment.

Conclusions

The results of both the reductive dechlorination enrichment culture experiment and the hydrogen and VFA monitoring bioassays combined with a comparison of carbon utilization in aerobic and anaerobic bioassays support the hypothesis that PBOC extracts can support reductive dechlorination. Enrichment cultures exhibited reductive dechlorination of PCE through *cis*DCE in the pyrophosphate extract amended cultures and dechlorinated PCE through VC in the alkali PBOC extract amended cultures. In anaerobic bioassays, hydrogen was produced at levels consistent with those necessary to drive reductive dechlorination. Furthermore, the alkali extracts produced more H_2 compared to the pyrophosphate extracts. These observations are consistent with the results of the enrichment cultures in which complete reductive dechlorination of PCE through VC was demonstrated in the alkali extract amended cultures with approximately 50% of the PCE converted into VC. Hydrogen production in anaerobic bioassays and conversion of PCE to *cis*DCE/VC in the reductive dechlorination enrichment culture experiments provided two lines of evidence for the utilization of PBOC extracts as a carbon source/electron donor. Although little to no VFA production was observed in the bioassays amended with pyrophosphate and alkali PBOC extracts, future work should interrupt the relationship between the hydrogen utilizing microorganisms and the fermenting organisms to allow VFA accumulation in the bioassays and increase the understanding of the complex carbon fermentation pathway. Over 45 days the aerobic bioassays exhibited a decrease in aqueous organic carbon in all bioassays while the anaerobic bioassays only exhibited a decrease in aqueous organic carbon for 50% of the bioassays. The results indicated that anaerobic bioassays may require a longer incubation time to observe organic carbon utilization, and the percent of carbon utilized was similar between the aerobic and anaerobic bioassays (24-32%) for the anaerobic bioassays exhibiting a change in aqueous organic carbon. Overall, the results of this investigation provide a validated method to help in the evaluation the sustainability of reductive dechlorination at chloroethene-contaminated sites.

References

Bouwer, E. J. and P. L. McCarty (1983). "Transformations of 1-Carbon and 2-Carbon Halogenated Aliphatic Organic-Compounds under Methanogenic Conditions." Applied and Environmental Microbiology **45**(4): 1286-1294.

Bradley, P. M. and F. H. Chapelle (1996). "Anaerobic mineralization of vinyl chloride in Fe(III)-reducing, aquifer sediments." Environmental Science & Technology **30**(6): 2084-2086.

Butler, E. C. and K. F. Hayes (1999). "Kinetics of the transformation of trichloroethylene and tetrachloroethylene by iron sulfide." Environmental Science & Technology **33**(12): 2021-2027.

CH2MHill (2000). Draft supplemental remedial investigation for Site 12. Technical Report, CH2MHill.

Chapelle, F. H., S. K. Haack, P. Adriaens, M. A. Henry and P. M. Bradley (1996). "Comparison of E(h) and H-2 measurements for delineating redox processes in a contaminated aquifer." Environmental Science & Technology **30**(12): 3565-3569.

Chapelle, F. H., J. T. Novak, J. Parker, B. B. Campbell and M. A. Widdowson (2006). A Framework for Assessing the Sustainability of Monitored Natural Attenuation USGS Circular Series C-1303., United States Geological Survey.

Cuypers, C., T. Grotenhuis, J. Joziasse and W. Rulkens (2000). "Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments." Environmental Science & Technology **34**(10): 2057-2063.

Debruin, W. P., M. J. J. Kotterman, M. A. Posthumus, G. Schraa and A. J. B. Zehnder (1992). "Complete Biological Reductive Transformation of Tetrachloroethene to Ethane." Applied and Environmental Microbiology **58**(6): 1996-2000.

DiStefano, T. D., J. M. Gossett and S. H. Zinder (1991). "Reductive Dechlorination of High-Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis." Applied and Environmental Microbiology **57**(8): 2287-2292.

Fennell, D. E., J. M. Gossett and S. H. Zinder (1997). "Comparison of butyric kid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene." Environmental Science & Technology **31**(3): 918-926.

He, Q. and R. A. Sanford (2002). "Induction characteristics of reductive dehalogenation in the ortho-halophenol-respiring bacterium, Anaeromyxobacter dehalogenans." Biodegradation **13**(5): 307-316.

Holliger, C. and W. Schumacher (1994). "Reductive Dehalogenation as a Respiratory Process." Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **66**(1-3): 239-246.

Kelly, C. A. (2006). Evaluation of Anaerobic Biodegradation of Organic Carbon Extracted from Aquifer Sediment. Thesis. Blacksburg, VA, Virginia Tech.

Krumholz, L. R., R. Sharp and S. S. Fishbain (1996). "A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation." Applied and Environmental Microbiology **62**(11): 4108-4113.

Matteson, K. M. (2004). Investigating the Biostimulating Effects of ESO Addition to a TCE Contaminated Site. Thesis, Virginia Tech.

Maymo-Gatell, X., Y. T. Chien, J. M. Gossett and S. H. Zinder (1997). "Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene." Science **276**(5318): 1568-1571.

Maymo-Gatell, X., V. Tandoi, J. M. Gossett and S. H. Zinder (1995). "Characterization of an H-2-Utilizing Enrichment Culture That Reductively Dechlorinates Tetrachloroethene to Vinyl-Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis." Applied and Environmental Microbiology **61**(11): 3928-3933.

McCarty, P. L. and D. P. Smith (1986). "Anaerobic Waste-Water Treatment .4." Environmental Science & Technology **20**(12): 1200-1206.

Mohn, W. W. and J. M. Tiedje (1992). "Microbial Reductive Dehalogenation." Microbiological Reviews **56**(3): 482-507.

Parsons, F., P. R. Wood and J. Demarco (1984). "Transformations of Tetrachloroethene and Trichloroethene in Microcosms and Groundwater." Journal American Water Works Association **76**(2): 56-59.

Rectanus, H. V. (2000). Assessment of Intrinsic Bioremediation at a PCE-Contaminanted Site. Thesis. Blacksburg, VA, Virginia Tech.

Roberts, A. L., L. A. Totten, W. A. Arnold, D. R. Burris and T. J. Campbell (1996). "Reductive elimination of chlorinated ethylenes by zero valent metals." Environmental Science & Technology **30**(8): 2654-2659.

Smatlak, C. R., J. M. Gossett and S. H. Zinder (1996). "Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture." Environmental Science & Technology **30**(9): 2850-2858.

Vogel, T. M. and P. L. McCarty (1985). "Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl-Chloride, and Carbon-Dioxide under Methanogenic Conditions." Applied and Environmental Microbiology **49**(5): 1080-1083.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, E. K. Gorden, J. T. Wilson, B. H. Wilson, D. H. Kambell, P. Haas, R. N. Miller, J. E. Hansen and F. H. Chapelle (1998). Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. Washington, DC, U.S. EPA.

Wiedemeier, T. H., J. T. Wilson and D. H. Kambell (1996). Natural Attenuation of chlorinated aliphateic hydrocarbons at Plattsburg Air Force Base, New York. Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Groundwater, Dallas, TX, U.S. EPA, Washington, DC.

Yang, Y. R. and P. L. McCarty (1998). "Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture." Environmental Science & Technology **32**(22): 3591-3597.

Chapter 5. Evaluation of potentially bioavailable organic carbon at a chloroethene-contaminated site

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Abstract

Potentially bioavailable organic carbon (PBOC) extraction data was used as a tool to investigate the effect of reductive dechlorination on the extractable organic carbon from sediment at a chloroethene-contaminated site. PBOC is an operationally-defined laboratory method to estimate the amount of readily-biodegradable organic carbon associated with an aquifer matrix. Prior to the laboratory phase, ground-water geochemical parameters illustrated that the environment in the aquifer was amenable to reductive dechlorination, and the ground-water contaminant concentrations demonstrated that dechlorinating populations were active through the conversion of PCE to cis-1,2-DCE with intermittent VC observations. PBOC measurements of samples collected both inside and outside of the chloroethene plume demonstrated that aquifer sediment inside of the plume contained less extractable PBOC than aquifer sediment outside of the plume. Aerobic bioassays demonstrated that 12-54% of the organic carbon extracted was biodegradable. The PBOC extraction bioassays exhibited decreased biodegradability of extractable PBOC inside the plume compared to outside the plume. The results support the hypothesis that decreased levels of PBOC and decreased biodegradability of organic carbon may be expected inside a chloroethene plume as a result of long-term reductive dechlorination activity relative to PBOC levels outside the plume. Furthermore, these results support the application of the PBOC method in evaluating the sustainability of reductive dechlorination at chloroethene-contaminated sites.

Introduction

Microbially-mediated reductive dechlorination is often a significant natural attenuation process in chloroethene-contaminated ground-water systems where the presence of a source of organic carbon combined with reducing redox conditions support an active dechlorinating

population of microorganisms (Bradley 2000). The sustainability of microbially-mediated reductive dechlorination is thought to be controlled by the supply of organic carbon derived from several sources (Chapelle et al. 2006). For example, anthropogenic organic carbon present in the plume (e.g., petroleum hydrocarbon compounds in a mixed source) can locally drive redox conditions to favor reductive dechlorination and the sustainability of natural attenuation is determined by the supply of carbon in the source. At other sites where the ambient redox conditions favor anaerobic metabolism, microbially-mediated reductive dechlorination is thought to be sustained by natural organic carbon (NOC) present in the sediment impacted by chloroethenes or present in ground water that mixes with the plume (Chapelle et al. 2006). For this latter scenario, known as Type II sites (Wiedemeier et al. 1996), it follows that an assessment of the sustainability of reductive chlorination requires characterization of the amount of NOC present.

In the previous chapters, methods to extract bioavailable organic carbon were investigated using samples collected from aquifer and surface water systems. Single liquid extraction techniques using pyrophosphate and alkali extracting solutions removed 1-20% of the total organic carbon (TOC_s) associated with the surface-water sediment and 2-38% of the TOC_s in the aquifer sediment. Aerobic bioassay experiments showed that 25-55% of the extracted aqueous carbon was biodegraded to carbon dioxide with 45 days of inoculation using a mixed culture of microorganisms. Based on the results of the single extractions and additional multiple extraction experiments, potentially bioavailable organic carbon (PBOC) was proposed as a quantitative measure for the long-term sustainability of reductive chlorination at Type II sites.

Additional bioassay experiments confirmed that extracted carbon or PBOC biodegraded in an anoxic environment, producing volatile fatty acids and molecular hydrogen. Furthermore, laboratory experiments conducted with an enrichment culture amended with tetrachloroethene (PCE) demonstrated that PCE was reduced to vinyl chloride with PBOC as the sole carbon source. Based on the result that PBOC supports reductive dechlorination in enrichment cultures, it is hypothesized that, given long-term exposure to chloroethenes in a reducing environment, reductive dechlorination activity will decrease the quantity of extractable PBOC in the aquifer sediment over time at a chloroethene-contaminated site.

To address this hypothesis, PBOC experiments were conducted using aquifer sediment samples collected at a PCE-contaminated site. Laboratory microcosm experiments, field

parameters, and chloroethene concentrations in the ground water demonstrated that microbially-mediated reductive dechlorination is active at the site. The objective of the study was to compare differences in extractable natural organic carbon using the PBOC extraction technique for samples collected inside and outside of the chloroethene-contaminated plume. As an additional step, bioassays were constructed to determine the extent to which extracted organic carbon biodegrades under aerobic conditions for each extraction. Carbon utilization and CO_2 evolution were monitored over time, and the results were used to compare differences between PBOC derived from sediment exposed to chloroethenes relative to that extracted from PBOC background samples.

Materials and Methods

Site Background

The Naval Amphibious Base Little Creek (NABLC) Site 12 is a former laundry/dry cleaning facility located in Virginia Beach, VA (Figure 5-1). From 1973 to 1978 dry cleaning waste containing PCE and other organic byproducts was disposed of through a manhole that fed into a storm sewer catch drain. An estimated 200 gallons of PCE and 1100 gallons of soaps, sizing, and dyes were discharged into the storm sewer (CH2MHill 2000). In preparation for the construction of a new base commissary, the main portion of the storm sewer was removed in 1987 and the remaining portion was removed in 1992. An investigation of the sewer system revealed ground water intrusion from a manhole and through a sewer line segment with inflow rates of 3-4 gpm from the manhole and 13 gpm from the leaky sewer segment (CH2MHill 2000). Site investigations began in 1984 and continued through March 2004 when the final feasibility study was completed and the record of decision was issued.

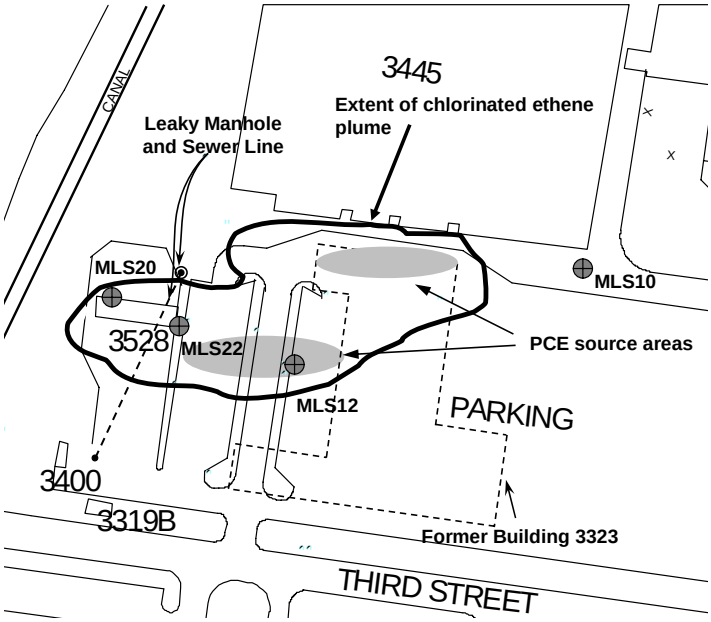

Figure 5-1. Locations of multi-level well samplers (MLS), leaky manhole, and sewer line at NAB Little Creek Site 12.

Chloroethene contamination at NABLC Site 12 is limited to the Columbia Aquifer, the upper-most hydrostratigraphic unit. The unconfined aquifer is approximately 20-25 feet thick and is comprised of Pleistocene deposits that are characterized by well-sorted, coarse sand with dispersed lenses of clay, silt, and gravel (CH2MHill 2000). Below the Columbia Aquifer is the Yorktown confining unit which separates the Columbia Aquifer from the Yorktown-Eastover Aquifer. The Yorktown confining unit is approximately 30 to 40 feet thick and is comprised of gray clay, silt, and fine sand.

The ground-water flow pattern in the Columbia aquifer at Site 12 is controlled by the leaky sewer system that is located 9 ft below ground level. Ground-water velocity is approximately 0.47 ft/day to the west in the Columbia aquifer at Site 12. Ground-water discharges to the sewer system at a rate of 16 gpm (CH2MHill 2000). Following dissolution of PCE into the ground water from the residual source, chloroethenes are subject to transformation by microbial reductive dechlorination. The travel time from the source zones to the sanitary sewer line is insufficient to completely eliminate chloroethenes. However, data demonstrated that the contaminants are degraded prior to discharge from the facility (CH2MHill 2000).

Ground-water Parameter Analyses

For this study, ground-water parameters at NABLC Site 12 were measured using multi-level sampling (MLS) wells. MLS well design provided the ability to measure a vertical distribution of a constituent by sampling ground water at seven discrete depths at each well. All ground-water samples were collected in 40 mL volatile organic analysis vials with the exception of hydrogen gas samples. Chloroethene samples were collected and analyzed using the method described in (Rectanus 2000). Aqueous sulfate was analyzed using ion chromatography. Ground-water samples for methane measurements were collected and analyzed by CH2MHill (2000).

Sediment Samples

Sediment sampling locations selected to compare PBOC extractions inside and outside of the plume were adjacent to MLS wells with known ground-water parameters (See Figure 5-1). MLS10 provided the sample from outside and up gradient of the plume. Three locations within the plume were sampled: MLS12, MLS20, and MLS22. MLS12 was identified as a PCE contaminant hot spot while MLS20 contained a factor of 10 less PCE and TCE compared with MLS12. Multi-level sampling well MLS22 provided ground water and sediment adjacent to the leaky sewer line. Aquifer sediment samples were collected with a Geoprobe unit and stored in acetate liners. All samples were kept at 4°C until analyzed.

PBOC Extractions

PBOC extractions followed the method outlined in Chapter 3 where dried, sieved aquifer sediment was combined with an extracting solution using a solution:sediment ratio of 2:1. As in Chapter 3, the PBOC is operationally defined by a five step extraction process where three 24-hour extractions with 0.1% pyrophosphate are followed with a 24-hour 0.5 N NaOH extraction followed by an additional 24-hour 0.1% pyrophosphate extraction. The sum of the first three 0.1% pyrophosphate extractions were reported as the pyrophosphate extract (mg/kg) and represent the loosely-extractable organic carbon associated with the sediment. Similarly, the final two extractions, where a 0.5 N NaOH solution was followed by 0.1% pyrophosphate solution, were reported as the alkali extract (mg/kg) and represent the more strongly-associated organic carbon associated with the sediment.

PBOC Bioassays

Aerobic bioassays were constructed using the method in Chapter 3. The inoculum used in the aerobic bioassays consisted of a consortium populated with facultative soil microbes. For inoculum extraction and growth methods, see Chapter 3. Aerobic bioassays were sampled every two weeks. Aqueous TOC samples were taken from the bioassays to document organic carbon utilization. To substantiate biogenic activity, headspace CO_2 was monitored.

The quantity of carbon utilized in the bioassays was operationally defined as the maximum decrease in TOC_{aq} concentration and statistically supported by the non-parametric Kruskal-Wallis one-way analysis of variance based on ranks. All statistical tests were run with the NCSS software package (Kaysville, Utah) using α=0.05. The statistical similarity between the medians from the triplicate TOC_{aq} values for each time point was evaluated. The statistically dissimilar medians were compared to the stabilization in incremental headspace CO_2 production to verify maximum decrease in aqueous carbon utilization.

Analytical Methods

Headspace CO_2 concentrations in the bioassays were determined with a gas chromatograph using a thermal conductivity detector (TCD, Shimatzu GC-14A). Aqueous organic carbon (TOC_{aq}) content for extraction and aerobic bioassay samples was analyzed via wet persulfate oxidation (Techmar Dohrmann DC-80). The solid-phase organic carbon content (TOC_s) was determined by elemental analysis (ThermoFinnigan Flash EA 1112). Soft carbon measurements followed the method outlined in Chapter 3 that was adapted from Cuypers et al. (2000).

Results and Discussion

To establish the relationship between PBOC in aquifer sediment and reductive dechlorination at a chloroethene-contaminated site, this study consisted of three parts. First, reductive dechlorination activity at the chloroethene site was assessed based on the ground-water geochemical and contaminant parameters. PBOC extractions on aquifer sediment from inside and outside of the plume were then evaluated in terms of the quantity of extractable organic carbon with respect to ground-water parameters. In the third component of the study, aerobic bioassay experiments were conducted to evaluate the extent to which PBOC extracts biodegrade.

Ground-water Geochemical and Contaminant Parameters

Ground-water samples were analyzed for constituents that indicated a positive environment for reductive dechlorination activity. The constituents investigated were either used or produced through terminal electron accepting processes driven by microorganisms under reduced conditions. These redox indicators consisted of an electron acceptor (sulfate) and an end product (methane). Overall, the redox indicators (Figure 5-2) suggested that the aquifer's terminal electron accepting processes differed with depth. In the upper portion of the aquifer, sulfate reduction and methanogenesis appeared to be prevalent. In the deep portion of the aquifer, methanogenic conditions dominated. Most importantly, these results showed that the redox environment at NABLC is amenable to reductive dechlorination. With respect to differences in the redox environment due to the presence of the plume, ground-water samples from outside of the plume (MLS10) followed the same general trend as ground-water samples from inside the plume (MLS12, MLS20, MLS22). There were slight differences, however, in the quantities of redox indicators as a result of the contamination. The levels of sulfate were higher in the upper portion of the aquifer at MLS10 compared against MLS20 and MLS22 which indicated that more sulfate was consumed inside the plume than outside. Similarly, methane levels inside the plume were elevated in comparison to outside of the plume which indicated more reduced conditions inside the plume.

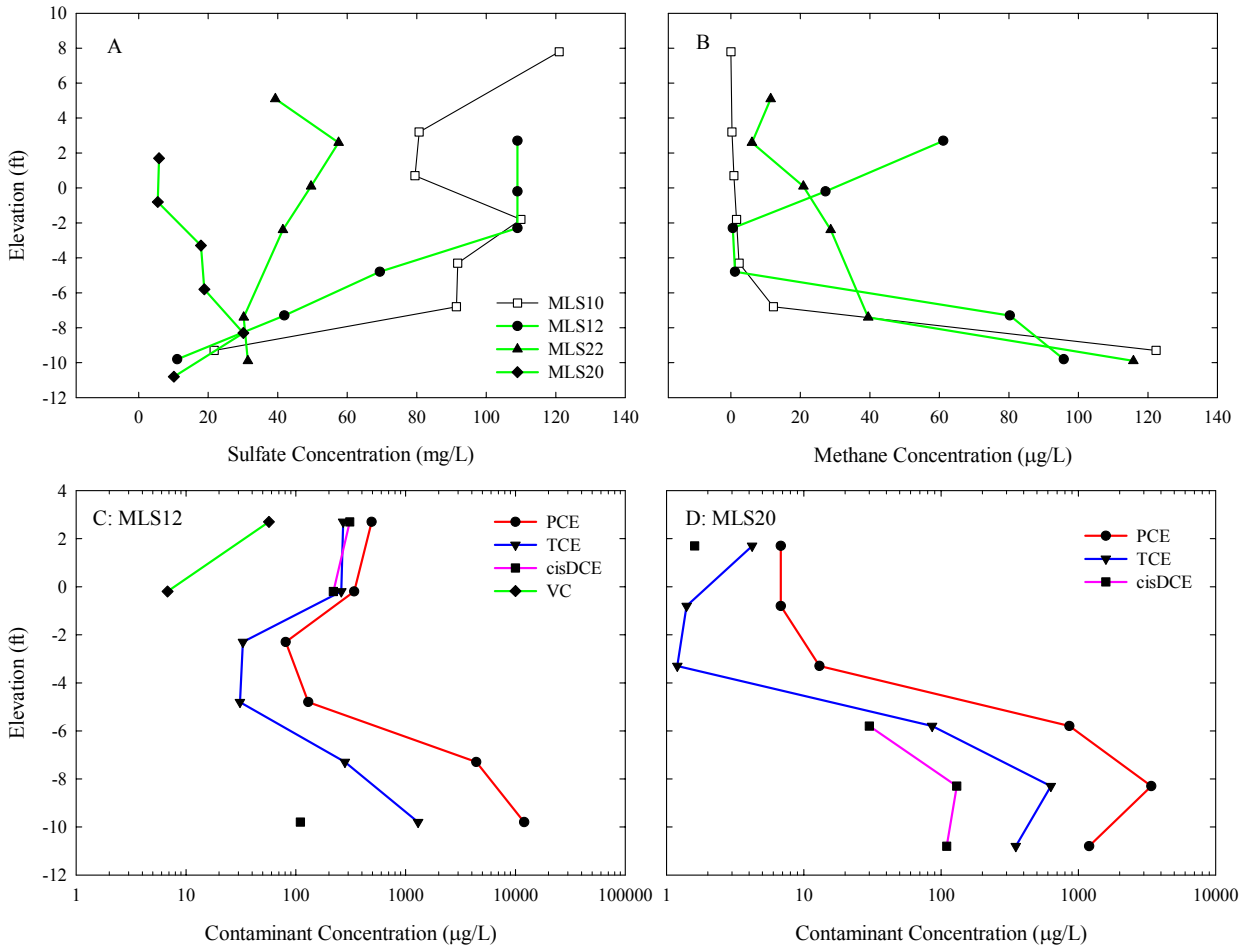

Figure 5-2. Summary of ground-water redox parameters and contaminant concentrations from July 1999 at NABLC. For (A) Sulfate concentrations and (B) Methane concentrations, each graph illustrates the distribution of the redox parameter over the depth of the multi level sampler (MLS) for MLS10, MLS12, MLS22, and MLS20. For (C) MLS12 and (D) MLS20, the chloroethene-contaminant concentrations are shown over the depth of MLS. Depth is shown in terms of elevation with respect to mean sea level.

Inside the plume, reductive dechlorination progressed readily from PCE to *cis*-1,2-DCE. Transitory VC observations suggested complete dechlorination does not occur throughout the site but in isolated pockets. Specifically the contaminant profile for MLS20 (Figure 5-2D) exhibited increased contaminant concentration for PCE, TCE, and *cis*-1,2-DCE contamination with depth. MLS22 exhibited the same trend in contaminant concentrations as MLS20 (data not shown). For MLS12, the PCE, TCE, and *cis*-1,2-DCE concentrations were highest in the shallow and deep depths but were lower at mid-level depths. VC observation was random throughout the wells and over the depths. In MLS22 and MLS20, no VC was detected. In

MLS12, VC was detected at the shallow depth. Supported by the ground-water parameters, the observations of PCE daughter products show that reductive dechlorination activity at NABLC Site 12 is active through *cis*-1,2-DCE with intermittent VC observations.

PBOC Extractions

As listed in Table 5-1, the pyrophosphate extractions (series of 3 0.1% pyrophosphate extractions) removed more organic carbon than the alkali extractions (base extraction followed by 0.1% pyrophosphate extraction) for each MLS sample location and depth. Furthermore, the quantity of extracted organic carbon increased between the shallow depth (8-10 ft) and deep depth (20-22 ft) for both the pyrophosphate and alkali extractions. However, alkali extracts decreased in extractable carbon at MLS0 for the 10-12 ft depth and at MLS12 for the 16-19 ft depth. For the MLS22 sediment samples, the organic carbon did not increase as the sample depth increased for the mid-level samples. This pronounced difference in extractable organic carbon may be a result of the location of MLS22 with respect to the leaky sewer line. During transient high flows, the influx of sewage may contribute to the increased quantity of extractable organic carbon in the 8-10 ft sample.

Table 5-1. PBOC Pyrophosphate and alkali extractions (mg carbon /kg sediment) at each depth for the sediment sampling locations of MLS10, MLS12, MLS20, and MLS22.

Sample Location	Extraction	TOC_{aq} Concentration (mg/kg) per Depth			
		8-10 ft	10-12 ft	16-19 ft	20-22 ft
MLS10	Pyrophosphate	39.85	34.43	149.59	324.23
	Alkali	13.78	7.47	26.11	111.61
MLS12	Pyrophosphate	15.46	34.11	33.24	142.78
	Alkali	3.01	9.45	1.83	51.77
MLS20	Pyrophosphate	6.13	9.61	29.64	182.23
	Alkali	2.25	2.80	6.49	51.01
MLS22	Pyrophosphate	62.05	26.31	35.88	268.03
	Alkali	25.99	6.06	4.80	55.56

With the quantity of extracted organic carbon being lower in the shallow depths (8-10 ft, 10-12 ft, and 16-19 ft samples) in comparison to the deep depth (20.5-22 ft samples), the increase in extractable organic with depth corresponded to textural transitions of the sediment. The shallow depth samples contained brown coarse sand with the solid-phase organic carbon content (TOC_s) ranging from 20-150 mg/kg and the soft organic carbon content between 52-60% of the TOC_s. Conversely, deep depth samples were comprised of well sorted sand with grey silt and clay

dispersed throughout the sample. The TOC_s ranged between 100-1200 mg/kg and the soft organic carbon content spanned 72-92% of the TOC_s. Based on the differing types of aquifer, the extractable organic carbon increased with depth and TOC_s.

When the PBOC values for all depths and all well locations are plotted against their TOC_s values (Figure 5-3), the outside of the plume values are larger than the inside of the plume values. Furthermore, the power regression through the data illustrates the difference between the inside and outside of the plume PBOC values. Given a solid-phase TOC_s of 100 mg/kg, the outside of plume would produce a total PBOC of 69% TOC_s while the inside of the plume would produce a total PBOC of 41% TOC_s. These results show that regardless of sediment depth the unconfined Columbia aquifer demonstrates the same trend of decreased extractable PBOC inside of the plume compared to outside of the plume.

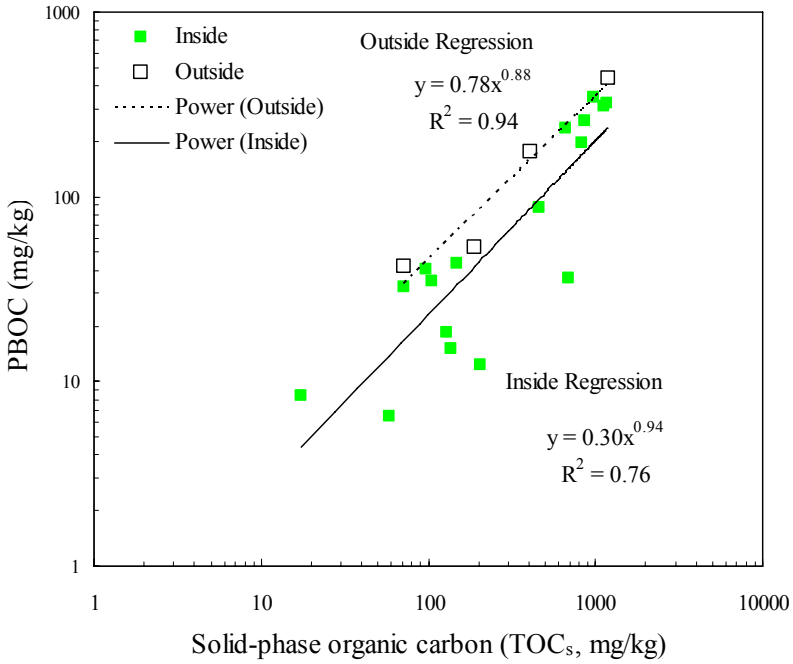

Figure 5-3. Extracted aqueous carbon concentration (mg/kg) as PBOC for the pyrophosphate and alkali extractions plotted against the solid-phase organic carbon (TOC_s, mgkg) over all depths for MLS10, MLS12, MLS20, and MLS22.

For the shallow depth, Figure 5-4 illustrates that the quantity of extractable organic carbon from pyrophosphate and alkali extractions outside the plume (MLS10) was higher than inside of the plume (MLS12 and MLS20). However, MLS22, also inside the plume, contained more pyrophosphate and alkali PBOC than MLS10. The data from MLS10, MLS12 and MLS20

support the hypothesis that PBOC extractions contained less carbon inside of the plume. This difference is thought to be due to consumption of the PBOC as a carbon source in the zone of active reductive dehalogenation. Although the data from MLS22 seem to contradict the hypothesis, the location of the shallow MLS22 sediment samples adjacent to the leaky sewer line was thought to increase the quantity of extractable organic carbon at MLS22 shallow. For the alkali extractions, the MLS10 extractions removed more carbon than the MLS12 and MLS22 extractions. The alkali extractions at MLS12 and MLS20 removed similar quantities of organic carbon which suggests that the sewer does not impact the lower depth at MLS22. With regard to observed reductive dechlorination at this shallow depth, ground-water contaminant profiles at MLS12, MLS20, and MLS22 indicated active reductive dechlorination by the presence of TCE and *cis*-1,2-DCE with the intermittent presence of VC. Overall, the results of the PBOC extractions were supported by the ground-water constituents and indicate utilization of the organic carbon associated with the sediment within the plume.

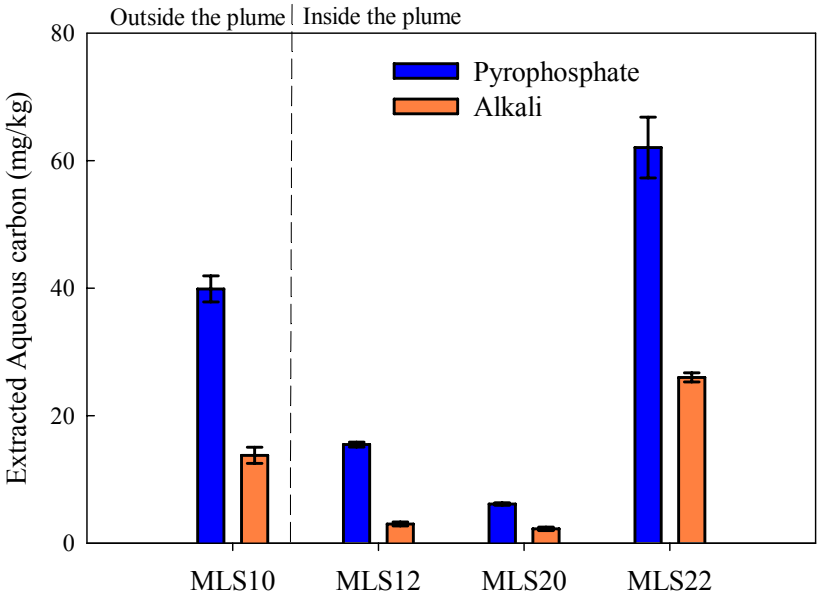

Figure 5-4. Extracted aqueous carbon concentration (mg/kg) for the pyrophosphate and alkali extractions at the shallow depth (8-10 ft) for MLS10, MLS12, MLS20, and MLS22.

At 20-22 ft depth shown in Figure 5-5, the quantities of pyrophosphate and alkali PBOC were higher outside of the plume (MLS10) when compared to inside of the plume (MLS12, MLS20, and MLS22). Furthermore, the pyrophosphate PBOC extraction at MLS12 was the lowest and this could be a result of high contaminant loading at this location near the source

zone. The alkali PBOC extractions inside the plume not only removed less organic carbon than outside the plume but also removed statistically similar quantities of organic carbon within the plume. Since both the shallow and deep sediment extractions produced similar results, this trend suggests similar alkali carbon utilization inside of the plume. As with the shallow PBOC extractions, the deep PBOC extraction results show increased carbon utilization inside of the plume compared to outside of the plume. The ground-water contaminant profiles in the deep depths inside the plume also showed production of PCE daughter products which indicated the contamination impacts the quantity of organic carbon removed by the PBOC extractions.

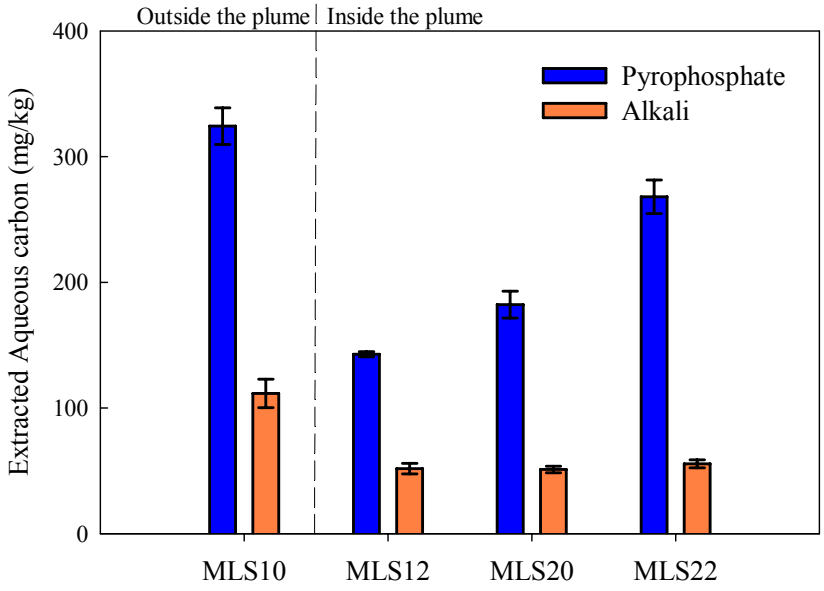

Figure 5-5. Extracted aqueous carbon concentration (mg/kg) for the pyrophosphate and alkali extractions at deep depth (20-22 ft) for MLS10, MLS12, MLS20, and MLS22.

Bioassay Experiments

Aerobic Bioassays

In Figure 5-6, a summary of the aerobic bioassay results for all depths across all well locations is shown. For all the data, 50% of the bioassays did not exhibit TOC utilization (as shown on the x-axis). These results show that when the extracted organic carbon was less than 3 mg/L, no biodegradability was seen in the bioassay. This data suggest that $TOC_{aq} < 3$ mg/L is a TOC threshold for observing carbon utilization in the bioassay method. Most importantly, the PBOC extracts from outside of the plume demonstrated biodegradability in 87.5% of the

bioassays while the PBOC extracts from inside of the plume only exhibited biodegradability in 41% of the bioassays. Overall, these results show that the extractable PBOC outside of the plume was more biodegradable than inside of the plume.

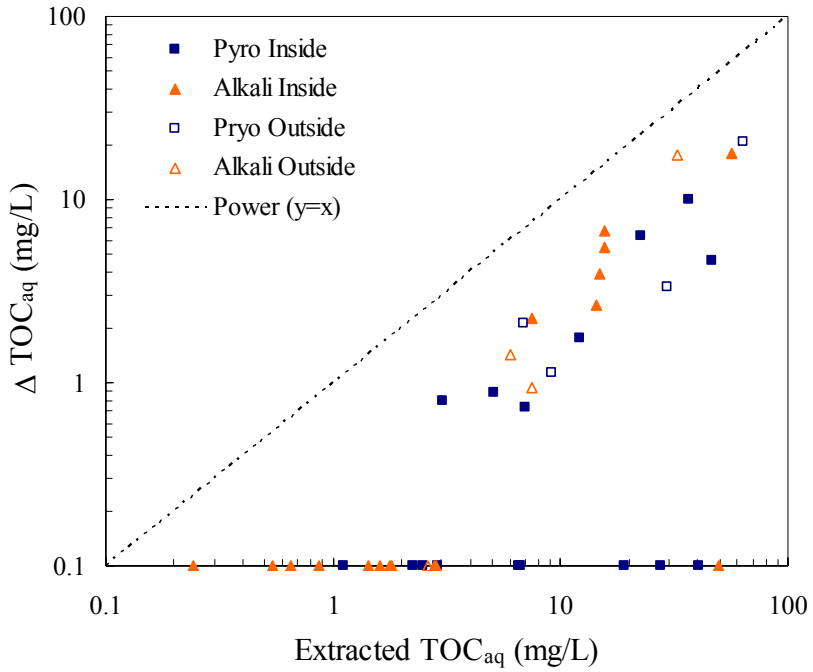

Figure 5-6. Summary of the of aerobic bioassay results in terms of carbon utilized (ΔTOC_{aq} mg/L) versus extracted TOC_{aq} concentration (mg/L) for the pyrophosphate and alkali extracts across all depths at MLS10, MLS12, MLS20, and MLS22. Due to the use of the log-log scale, all bioassays that exhibited no utilization of TOC_{aq} are shown on the x-axis at an artificial value of ΔTOC=0.1 mg/L.

The aerobic bioassays showed that the pyrophosphate extractions from outside of the plume (MLS10) contained more biodegradable carbon than the pyrophosphate extractions from inside of the plume (MLS20). For the shallow (8-10 ft) and mid-level (10-12 ft) samples, Table 5-2 shows that the MLS10 bioassays exhibited 1.1 and 2.1 mg/L TOC utilization at the shallow and mid-level depths, respectively. In comparison, the low quantities of TOC_{aq} extracted from the shallow and mid-level MLS20 samples were at the detection limit and resulted in negligible observed carbon utilization. For the deep samples, the pyrophosphate MLS10 extractions removed two times more organic carbon than the pyrophosphate MLS20 extractions, and the pyrophosphate MLS10 bioassays exhibited two times more TOC utilization than the pyrophosphate MLS20 bioassays. Overall, the pyrophosphate extract bioassays showed less biodegradable PBOC within the plume at all depths compared to outside of the plume.

For the alkali extractions, the aerobic bioassays also showed that extractions from outside of the plume (MLS10) contained more biodegradable carbon than extractions from inside of the plume (MLS20). In the shallow samples, the alkali MLS10 bioassays exhibited similar TOC utilization to the pyrophosphate MLS10 bioassays even though the quantity of carbon extracted by the alkali MLS10 extractions was lower than the quantity of carbon extracted by the pyrophosphate MLS10 extractions. The mid-level, alkali MLS10 bioassay exhibited negligible carbon utilization as a result of the low quantity of extracted carbon. Similarly, the alkali MLS20 bioassays at the shallow and mid-level depths resulted in negligible carbon utilization. For the deep samples, the pyrophosphate MLS10 extractions removed two times more organic carbon than the pyrophosphate MLS20 extractions, and the pyrophosphate MLS10 bioassays exhibited two times more TOC utilization than the pyrophosphate MLS20 bioassays. Overall, the alkali extract bioassays showed PBOC consumption inside of the plume, and lower levels of TOC utilization were observed in the alkali extract bioassays in comparison to the pyrophosphate extract bioassays.

Table 5-2. Summary of aerobic bioassay results in terms of carbon extracted (mg/L), carbon utilized (mg/L) and % carbon utilized for the pyrophosphate and alkali extractions at MLS10 and MLS20.

		Pyrophosphate Extraction		Alkali Extraction	
Sample	Depth	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)
MLS10	8-10 ft	7.87	1.12	3.95	1.42
MLS10	10-12 ft	6.88	2.11	2.62	0*
MLS10	20.5-22 ft	64.17	20.61	32.58	17.50
MLS20	8-10 ft	2.26	0*	1.61	0*
MLS20	10-12 ft	2.89	0*	1.79	0*
MLS20	22 ft	36.53	10.06	15.74	6.80

* No statistical aqueous TOC decrease was observed in bioassays

The comparison between the outside and inside of plume revealed that sediment extractions from outside of the plume produced more extractable carbon and more biodegradable organic carbon than sediment extractions from inside of the plume. These results show that the PBOC method can estimate the relative quantities of biodegradable organic carbon outside and inside of the plume and provides means to assess the sustainability of reductive dechlorination at the site.

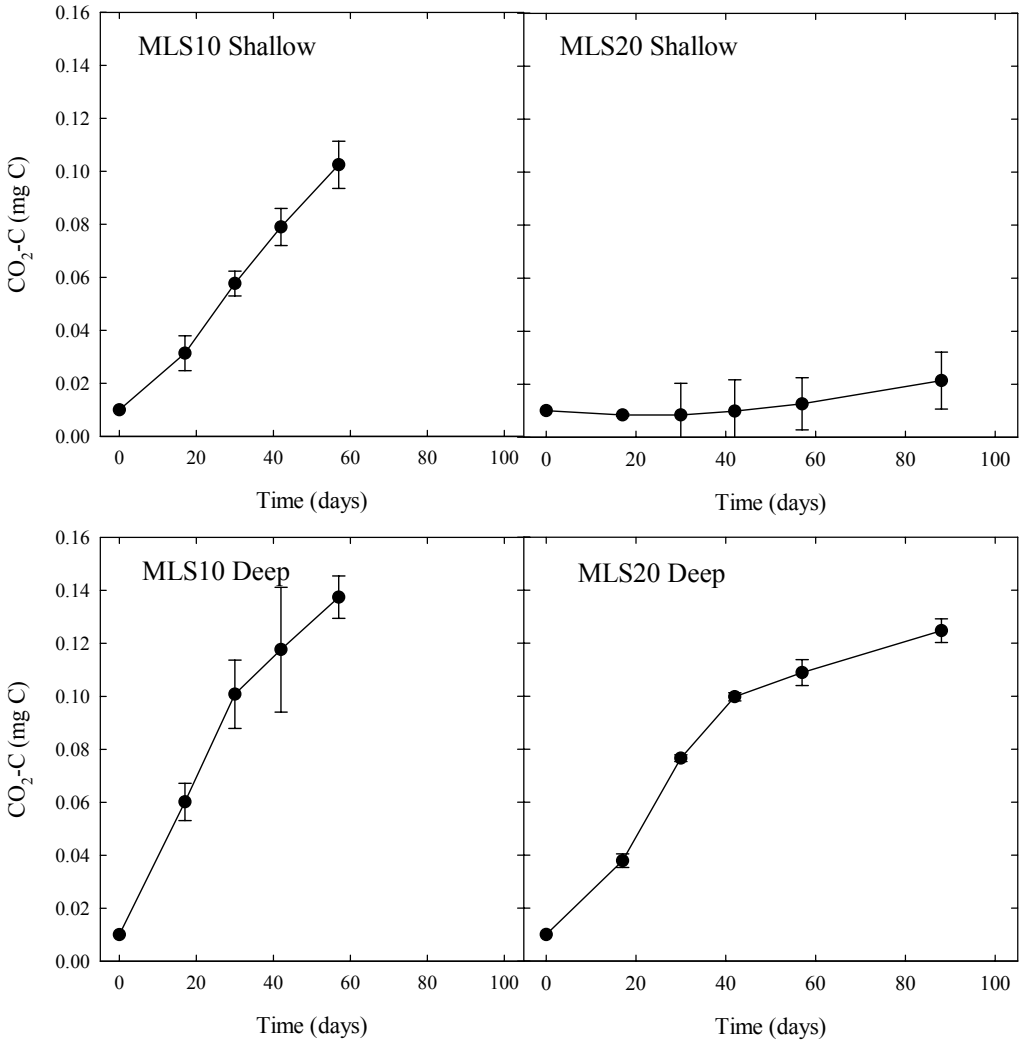

Figure 5-7. Cumulative headspace CO_2 generation shown as mg C produced per bioassay sample for pyrophosphate extract bioassays of MLS10 and MLS20 at the shallow and deep depths.

To support the organic carbon biodegradability observed in the bioassays by TOC_{aq} decrease, the headspace CO_2 results are presented. Figure 5-7 illustrates that the MLS10 bioassays produced more CO_2 than the MLS20 bioassays which agrees with the aqueous TOC utilization data. The CO_2 data further support that the PBOC extractions outside of the plume (MLS10) contained more biodegradable organic carbon than the PBOC extractions inside of the plume (MLS20). Figure 5-7 shows the results only of the pyrophosphate extraction bioassays; the alkali extractions bioassays have the same trends but are not shown. For the shallow MLS10 bioassays, the utilization of TOC resulted in accumulation of over 0.1 mg CO_2-C as headspace

CO_2 whereas the shallow MLS20 CO_2 results supported the negligible utilization of TOC_{aq}. The low levels of headspace CO_2 (0.0007 mg CO_2-C) produced at day 14 in the shallow MLS20 bioassay were likely the result of endogenous respiration from the added soil culture. Subsequent CO_2 production was attributed to microbial re-growth which was seen at day 53. For the deep pyrophosphate extractions, MLS10 bioassays produced 0.016 mg more headspace CO_2-C than the MLS20 bioassays. Although the difference in headspace CO_2 production did not correlate with the difference in TOC utilization between the deep MLS10 and MLS20 bioassays, the CO_2 measurements do corroborate the biogenic use of TOC.

A carbon mass balance was not performed because solution pH was not taken at each time point during the experiment. Furthermore, since the system was well buffered, the precision of pH readings could not have resolved the capacity of the system to capture bicarbonate species and the mass balance would not have accurately reflected the situation. Therefore, the inconsistencies in CO_2 headspace concentrations between the MLS10 and MLS20 deep bioassays were not addressed.

Conclusions

Based on the previous results showing that PBOC supports reductive dechlorination in enrichment cultures (Chapter 4), it was hypothesized that reductive dechlorination activity within a chloroethene plume would decrease the quantity of extractable PBOC in the aquifer sediment. To address this hypothesis, experiments were conducted comparing the sediment-bound organic carbon inside and outside of the plume using samples from a PCE-contaminated site where reductive dechlorination activity was active through *cis*-1,2-DCE with intermittent VC observations. PBOC extractions at shallow and deep sample depths showed less carbon was extracted from samples inside of the plume compared to outside of the plume. These results supported the hypothesis that the quantity of organic carbon present in the sediment inside the plume was consumed by reductive dechlorination. Aerobic bioassays demonstrated that a greater fraction of PBOC inside of the plume biodegraded compared to PBOC outside of the plume. These results further supported the assertion that reductive dechlorination activity directly decreased the quantity and quality with respect to the degradability of extractable PBOC. Overall, these results indicated that the PBOC method may be useful in assessing the reductive dechlorination sustainability in the context of MNA.

References

Bradley, P. M. (2000). "Microbial degradation of chloroethenes in groundwater systems." Hydrogeology Journal **8**(1): 104-111.

CH2MHill (2000). Draft supplemental remedial investigation for Site 12. Technical Report, CH2MHill.

Chapelle, F. H., J. T. Novak, J. Parker, B. B. Campbell and M. A. Widdowson (2006). A Framework for Assessing the Sustainability of Monitored Natural Attenuation USGS Circular Series C-1303., United States Geological Survey.

Cuypers, C., T. Grotenhuis, J. Joziasse and W. Rulkens (2000). "Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments." Environmental Science & Technology **34**(10): 2057-2063.

Rectanus, H. V. (2000). Assessment of Intrinsic Bioremediation at a PCE-Contaminanted Site. Thesis. Blacksburg, VA, Virginia Tech.

Wiedemeier, T. H., J. T. Wilson and D. H. Kambell (1996). Natural Attenuation of chlorinated aliphateic hydrocarbons at Plattsburg Air Force Base, New York. Proceedings of the Symposium on Natural Attenuation of Chlorinated Organics in Groundwater, Dallas, TX, U.S. EPA, Washington, DC.

Chapter 6: Distribution of potentially bioavailable organic carbon extractions on aquifer sediments at chloroethene contaminated sites

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Abstract

In this study, the distribution of potentially bioavailable organic carbon (PBOC) was investigated across six contaminated sites. Aquifer sediment was collected at six chloroethene-contaminated sites that encompassed a wide range of hydrogeology, organic carbon content, and reductive dechlorination activity. PBOC was correlated quantitatively to total organic carbon (TOC_s) and soft carbon content of the sediment and qualitatively to the reductive dechlorination activity at the sites. From empirical expressions, PBOC comprised 30% of TOC_s and soft carbon consisted of 51% of TOC_s. Based on these correlations, a range for bioavailable organic carbon associated with the aquifer sediment was proposed where the upper bound consisted of soft carbon and the lower bound consisted of PBOC. When PBOC was separated into the pyrophosphate and alkali PBOC components, the correlations showed that at the sites with active reductive dechlorination pyrophosphate extractions removed 30% of the organic carbon while alkali extractions removed 12% of the organic carbon. At sites exhibiting minimal dechlorination, sediment with low TOC_s released more carbon with the pyrophosphate extractions while sediment with high TOC_s released more carbon with the alkali extractions. Both pyrophosphate and alkali extracts contained biodegradable organic carbon at active and minimal reductive dechlorination sites. At sites with active reductive dechlorination, the biodegradability of the pyrophosphate and alkali PBOC extracts was lower, which indicated an impact on the sediment-associated organic carbon due to reductive dechlorination. Overall, the variability in the quantity of organic carbon removed by the PBOC extractions correlated well with reductive dechlorination activity at a site.

Introduction

Chloroethenes have been observed at over 50% of the National Priorities List (NPL) sites in the United States (Butler and Hayes 1999). The chloroethenes, tetrachloroethene (PCE) and trichloroethene (TCE), are used as degreasers in industrial and military facilities and are also the most commonly used chemicals in dry cleaning because of their low flammability and high vapor pressures (Fetter 1999). However, through improper storage, handling, and disposal, PCE and TCE have been released into the environment and become ubiquitous ground-water contaminants. One biogenic degradation pathway, reductive dechlorination, occurs in anaerobic environments when microorganisms reduce chloroethenes by sequentially replacing chlorine with hydrogen in the molecular structure. Microbially-mediated reductive dechlorination of PCE and TCE produces the daughter products, *cis*-1,2-dichloroethene (*cis*-1,2-DCE), *trans*-1,2-dichloroethene (*trans*-1,2-DCE), vinyl chloride (VC), ethene, and ethane (Mohn and Tiedje 1992; Maymo-Gatell et al. 1997). The ability of microorganisms to intrinsically biodegrade chlorinated solvents prompted the consideration of monitored natural attenuation (MNA) as a remediation strategy at chlorinated solvent sites (EPA 1999).

MNA is a remediation technology applicable to chloroethenes that utilizes the in situ capabilities of the aquifer, such as dispersion, sorption, volatilization and biodegradation, to reduce the contaminant concentration (Chapelle and Bradley 1998; Wiedemeier et al. 1998). A principal component of MNA at chlorinated solvent-contaminated sites is often reductive dechlorination. Under suitable conditions, MNA can be an effective method for meeting a site-specific remedial action objective (RAO) at a site contaminated with chlorinated solvents. Two components of MNA are source control and long-term performance monitoring (EPA 1999). Chapelle et al. (2005) demonstrated this approach at a PCE-contaminated sites and documented the reduction in the ground-water concentrations of VC and other chloroethenes over a seven-year period following source remediation. Chapelle et al. (2003) refer to the reduction of plume size as distance of stabilization and the timeframe to meet the RAO at the point or plane of compliance as time of stabilization.

Because remediation timeframes using MNA may span decades at some sites, it is important to consider, evaluate, and predict the long-term sustainability of the natural processes that attenuate chlorinated solvent plumes, specifically reductive dechlorination. Chapelle et al. (2006) recently developed an approach to assessing the long-term sustainability of MNA and

demonstrated the approach at a chloroethene-contaminated site. Through the use of a deterministic mass and energy balance on electron donors and acceptors in a ground water, a range of estimates for the time of remediation under MNA can be quantified based on the mass of contamination and the energy required to completely transform contaminants to innocuous products (Chapelle et al. 2006). In some ground-water systems, the energy to drive and sustain reductive dechlorination is derived from natural organic carbon present in aquifer sediment inside the plume and the mass flux of carbon that mixes with the contamination. Therefore, quantifying the potentially bioavailable organic carbon (PBOC) in aquifer sediments is essential for estimating the long-term sustainability of reductive dechlorination at sites contaminated with chlorinated solvents.

In the previous three chapters, a method to measure PBOC was tested and evaluated using sediment samples and then demonstrated at a chloroethene-contaminated site. A technique was developed in which aquifer sediment samples are exposed to a series of liquid extractions to remove natural organic carbon associated with the aquifer sediment. Results of aerobic and anaerobic bioassays confirmed that extracted carbon biodegraded relative to control experiments. Under anaerobic conditions, H_2 was produced at levels necessary to maintain reductive dechlorination. Using the extractable PBOC as the sole electron donor, enrichment culture experiments exhibited reductive dechlorination of PCE through VC and established a relationship between the PBOC extractions and reductive dechlorination. The PBOC method was also applied to samples collected at a PCE-contaminated site with an active population of dechlorinating microorganisms to investigate the difference in extractable organic carbon inside and outside of a chloroethene-contaminated plume. Supported by the ground-water constituents, PBOC extractions and bioassays showed that less extractable organic carbon was present inside of the chloroethene plume than present outside of the plume. These research results indicated that the PBOC method may be useful in assessing the reductive dechlorination sustainability in the context of MNA.

Building on the work from Chapters 3-5, a study was undertaken to investigate the distribution of PBOC at six chloroethene-contaminated sites in the United States. It was hypothesized that PBOC was correlated to the degree to which microbially-mediated reductive dechlorination is present at a site. To evaluate this hypothesis, aquifer sediment samples were collected at six chloroethene-contaminated sites from hydrogeologically-diverse locations that

encompassed a wide range of organic carbon content and reductive dechlorination activity. Then, PBOC extractions and bioassays were run on the aquifer sediments. The results of the PBOC extractions were compared to the solid-phase total organic carbon and soft carbon content of the aquifer sediments as well as the reductive dechlorination activity at the sites. Pyrophosphate and alkali PBOC bioassays results were evaluated in terms of reductive dechlorination activity at the sites. Based on relationships in the above mentioned parameters, a range for bioavailable organic carbon associated with the aquifer sediment was proposed.

Materials and Methods

Study Sites

Sediment samples from six sites were used as test material for the PBOC multiple extraction and bioassay method. Sediment samples (See Table 6-1) were collected through collaborative efforts with the Southern Division of the Naval Facilities Engineering Command (NAVFAC), United States Geological Survey (USGS), CH2MHill, and the United States Navy. Samples were provided from two sites in semi-arid regions of the U.S. (DOD, CA and NAS, TX), one site in a glaciated region of the north-central U.S. (ACP, MN), and three sites in the Atlantic Coastal Plain (MCPR, SC, NABLC, VA, and NSB, GA). Aquifer sediment samples were collected with a traditional split-spoon drill unit and stored in Mason jars from the NAS, ACP, MCRP, and DOD facilities. Samples collected at the NABLC were taken with a Geoprobe unit and stored in acetate liners. The NSB sediment samples were collected using a hand auger and stored in Mason jars. After the receipt of the sediment samples at Environmental Engineering Laboratory at Virginia Tech, all samples were kept at 4°C until analysis.

Table 6-1 summarizes the site information, aquifer sediment characteristics, contaminant of concern and reductive dechlorination activity for each site used in this study. Reductive dechlorination activity was assessed using the Wiedemeier et al. (1998) classification system for chloroethene-contaminanted plumes. The Type I and Type II environments were designated as active dechlorination sites, and the Type III environments were labeled as sites with minimal reductive dechlorination activity. All sediment samples were taken from within the contaminant plume.

Table 6-1. Summary of site information, sediment characteristics and reductive dechlorination activity for aquifer sediment samples.

Site Name	Location	Aquifer Sediment Sample IDs	Sediment Characteristics	Contamination	Reductive Dechlorination
DOD/CA	Department of Defense facility, CA	10 CO 39 RW	Sandy	TCE	Minimal
		10 CO 41 RW	Sandy		
		10 CO 49 MW	Silty, sandy		
ACP	Anoka County Riverfront Park, MN	PESMW13A	Brown, Sandy	TCE	Minimal
		PESMW10B	Fine Sandy		
NAS	Dallas Naval Air Station, TX	799E152I	Brown, Sandy	TCE	Minimal
		799E115I	Brown, Silty sandy		
		799E151U	Brown, Sandy		
MCRP	Marine Corps Recruit Depot Parris Island, SC	PAI-45-MW21-SU	Brown, Sandy gravel	PCE and TCE	Active
		PAI-45-MW22-SU	Brown, Sandy gravel		
		PAI-45-MW22-SL	Grey, Silty sandy		
		PAI-45-MW21-SL	Grey, Silty sandy		
NABLC	Naval Amphibious Base Little Creek, VA	MLS10	Shallow depths: Sandy Deep depths: Grey, Silty sand	PCE	Active
		MLS12			
		MLS20			
		MLS22			
		MW06			
		MIP			
NSB	Naval Submarine Base Kings Bay, GA	Outcrop	Black silty sandy	PCE	Active
		KBA-13A	Sandy		
		5/11/2002	Grey Silty		

PBOC Extraction Method

Liquid extractions followed the method described in previous chapters where dried, sieved aquifer sediment was combined with an extracting solution using a solution:sediment ratio of 2:1. The PBOC extraction method consisted of the following sequential steps: three 24-hour extractions with 0.1% pyrophosphate, one 24-hour extraction with 0.5 N NaOH, and one 24-hour extraction with 0.1% pyrophosphate. For each PBOC extraction series, triplicate extraction

samples were run. The sum of the first three 0.1% pyrophosphate extractions were reported as the pyrophosphate extract (mg/kg) and represent loosely-extractable organic carbon associated with the sediment. Similarly, the final two extractions, where a 0.5 N NaOH solution was followed by 0.1% pyrophosphate solution, were reported as the alkali extract (mg/kg) and represent more strongly-associated organic carbon associated with the sediment.

PBOC Bioassay Methods

Aerobic bioassays were run using the method described in previous chapters. The inoculum used in the aerobic bioassays consisted of a soil microorganism consortium. Aerobic bioassays were sampled every two weeks for aqueous TOC and headspace CO_2 concentrations. Aqueous TOC samples were taken to document organic carbon utilization, and headspace CO_2 was monitored to substantiate biogenic activity.

To evaluate the biodegradability of the PBOC in the bioassay experiments, the maximum change in TOC concentrations over time was calculated. A statistical significant decrease in TOC_{aq} was determined with the non-parametric Kruskal-Wallis One-way Analysis of Variance test using a 95% confidence limit (alpha=0.05) (NCSS, Kaysville, Utah). To further support the operationally defined decrease in TOC_{aq}, the time point associated with the lowest TOC_{aq} concentration was compared to the time point where stabilization in headspace CO_2 production was observed.

Analytical Methods

Headspace CO_2 concentrations in the bioassays were determined with a gas chromatograph using a thermal conductivity detector (TCD, Shimatzu GC-14A). TOC_{aq} content in samples was analyzed via wet persulfate oxidation (Techmar Dohrmann DC-80). The solid-phase total organic carbon (TOC_s) content for the aquifer sediment was determined by elemental analysis (ThermoFinnigan Flash EA 1112). Soft carbon measurements followed the method outlined in described in previous chapters that was adapted from Cuypers et al. (2000).

Results and Discussion

PBOC Extractions

The PBOC (sum of the pyrophosphate and alkali PBOC extracts) was compared to the solid-phase TOC_s for all the sediment samples. Figure 6-1 illustrates that as solid-phase TOC_s increased, the PBOC also increased. Labeling the data from each site with the appropriate site name and reductive dechlorination status showed that the sites with relatively high levels of TOC_s and PBOC corresponded with observable levels of the reductive dechlorination activity. The active reductive dechlorination sites are shown in green circles while the sites exhibiting minimal reductive dechlorination are shown in red squares. The DOD site exhibited negligible reductive dechlorination and contained the least amount of TOC_s and PBOC. Conversely, the NSB site showed active reductive dechlorination of PCE through VC and ethene production while containing high levels of TOC_s and PBOC. While the NABLC and MCRP sites exhibited active reductive dechlorination, the rate and extent of reductive dechlorination was moderate in comparison to the NSB site. For the ACP site, the low levels of reductive dechlorination daughter product formation were seen in the ground water but little attenuation in the contaminant was observed over time. Excluding the NAS site data, Figure 6-1 shows that the reductive dechlorination activity was qualitatively correlated to the TOC_s and PBOC levels. The reductive dechlorination activity at NAS, however, is known to be limited although the site contains high levels of organic carbon. This limitation is a result of the redox condition in the aquifer that does not favor reductive dechlorination. Overall, the data trend showed a positive correlation between the solid-phase TOC_s, PBOC, and reductive dechlorination activity.

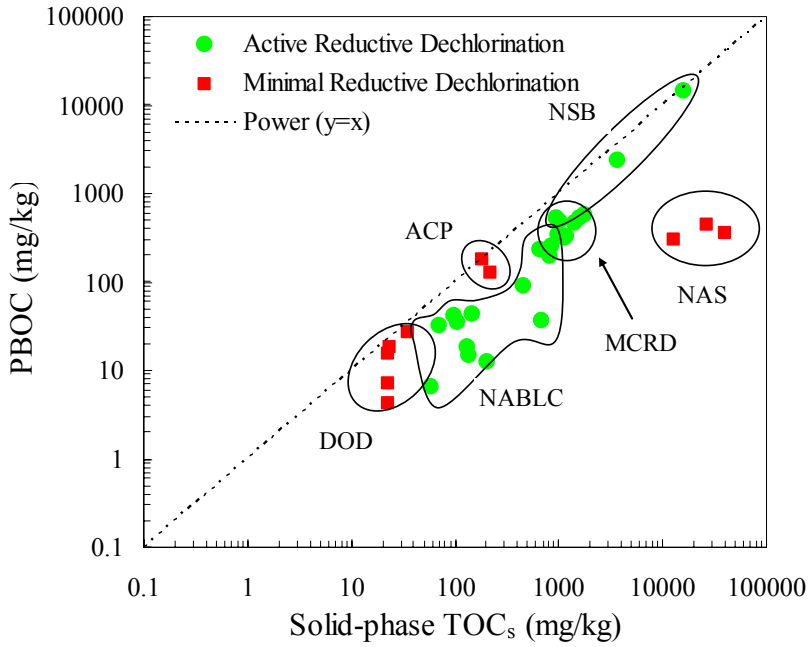

Figure 6-1. PBOC (mg/kg) versus the solid-phase total organic carbon (TOC_s, mg/kg) for the six study sites. Data is labeled and encircled for each site.

The TOC_s and PBOC data for NAS site did not follow the same trend as the other sites. When the NAS data are considered as outliers, the TOC_s and PBOC data fit a power function (Figure 6-2). The regression indicated that 32% of the TOC_s was extracted using the PBOC method (assuming 100 mg/kg TOC_s). Furthermore, the range of TOC_s for this correlation was applicable across the range of aquifer sediment used in this study. The strong correlation between PBOC and TOC_s resulted in an empirical expression to estimate the PBOC from an aquifer sediment sample with a known TOC_s.

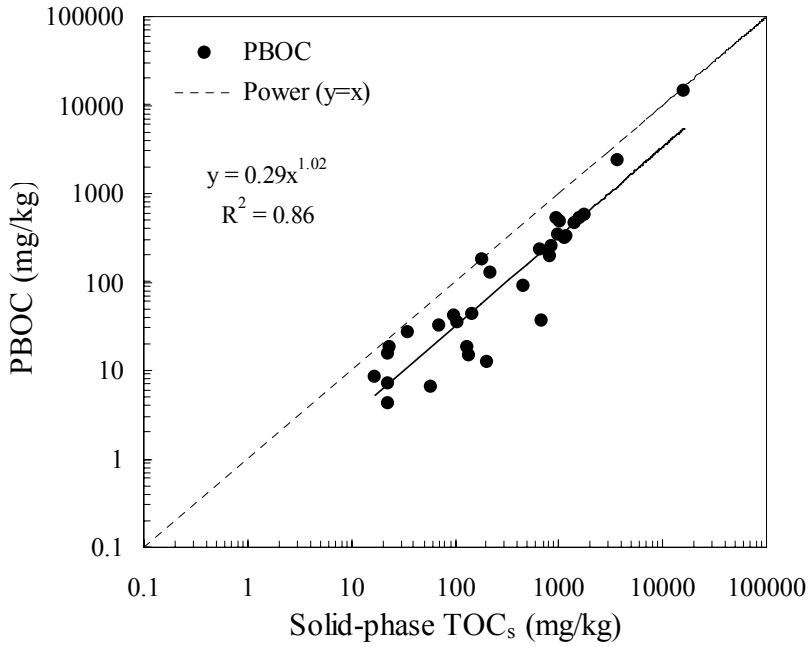

Figure 6-2. Summary of PBOC (mg/kg) versus the sediment total organic carbon (TOC_s, mg/kg) for five of the six study sites (the NAS data are omitted).

As discussed in Chapter 3, it was hypothesized that the soft carbon content of aquifer sediment provides the theoretical upper bound for the organic carbon available over extended periods of time (> 100 years) to subsurface microorganism communities. To address this hypothesis, soft carbon analyses were performed on all the aquifer sediment samples and the relationship between soft carbon and TOC_s are shown in Figure 6-3. As TOC_s increased, the soft carbon content of the sediment also increased. The soft carbon content followed the same trend as PBOC with respect to TOC_s. This trend, however, was only seen in sites exhibiting active reductive dechlorination because the majority of the sites exhibiting minimal reductive dechlorination contained negligible soft carbon content. Only one sediment sample from ACP contained measurable soft carbon. The lack of soft carbon for the DOD and ACP sites further supported the hypothesis that soft carbon is the primary contributor to the pool of bioavailable organic carbon that support subsurface microorganisms. As with PBOC, the regression provided an empirical expression from which to estimate soft carbon content of a sample when TOC_s is known. For the active reductive dechlorination sites, 58% of the TOC_s consisted of soft carbon (assuming 100 mg/kg TOC_s).

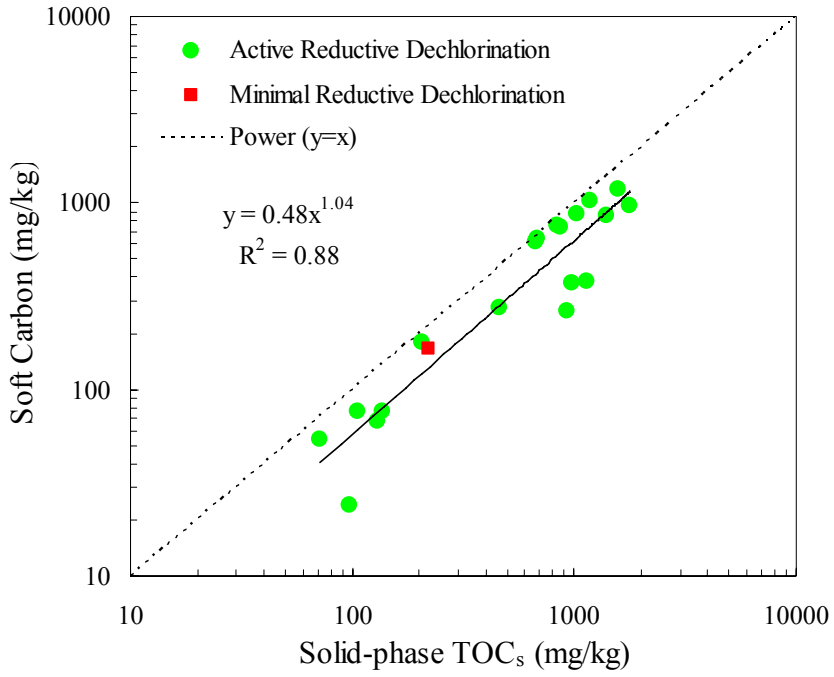

Figure 6-3. Soft carbon content (mg/kg) versus the sediment total organic carbon (TOC_s, mg/kg) for aquifer sediment containing soft carbon.

To further investigate the notion that soft carbon content served as a potential upper bound for bioavailable organic carbon associated with the aquifer sediment, PBOC was plotted against soft carbon content (Figure 6-4). A moderate correlation between PBOC and soft carbon was observed where R^2=0.60. Although error in the soft carbon and extraction analyses resulted in two data points where the PBOC extracts were greater than the soft carbon content, the relation between the soft carbon and the total PBOC suggested that 43% of the soft carbon was extracted by the PBOC method (assuming 100 mg/kg TOC_s). If the soft carbon and PBOC extracts originated from the same organic carbon pool, then the theoretical contribution of PBOC from the soft carbon can be estimated from the regressions in Figures 6-2 and 6-3. From the correlations above, 32% of TOC_s was extracted by the PBOC method and 58% of TOC_s consisted of soft carbon. The theoretical contribution would predict 55% of the soft carbon coming from the PBOC extractions. The theoretical prediction of 55% over predicted but was similar to the regression expression of 43%. Based on the similarity of the empirical expressions between TOC_s, PBOC, and soft carbon, further support was provided for the hypothesis that soft carbon provides the upper bound in bioavailable organic carbon associated with the sediment. From these correlations, a range for bioavailable organic carbon associated with the aquifer

sediment is proposed where the upper bound is the soft carbon and the lower bound is the total PBOC. However, it should be noted that the methods in which organic carbon becomes bioavailable are not understood and require investigation.

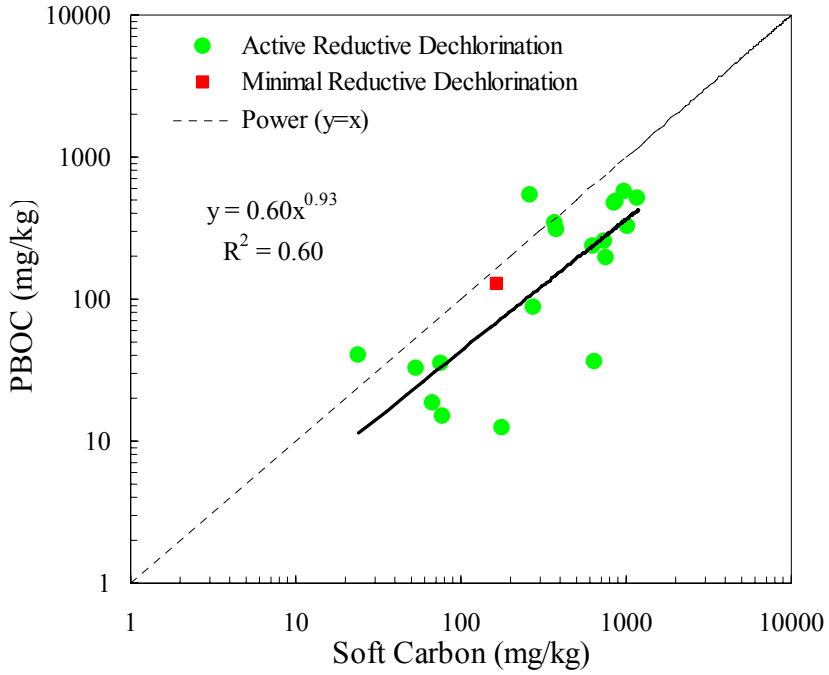

Figure 6-4. PBOC (mg/kg) versus the soft carbon content (mg/kg) for aquifer sediment for the four of six study sites (the NAS and DoD data are omitted).

Pyrophosphate and Alkali PBOC Extractions

Per the operationally definition, PBOC was divided into the pyrophosphate and alkali fractions, which represented the loosely-bound organic carbon and the more strongly-bound organic carbon, respectively. With the separation of the pyrophosphate and alkali extractions, their relative contribution to the PBOC and subsequently to TOC_s and soft carbon content was investigated. In Figure 6-5, the pyrophosphate and alkali extracts are shown with respect to the TOC_s for the active reductive dechlorination sites and for the minimal reductive dechlorination sites. In the active sites (Figure 6-5A), the pyrophosphate extractions removed more organic carbon than the alkali extractions. Based on 100 mg/kg TOC_s, the regressions indicated that the pyrophosphate extractions would release 19% of TOC_s while the alkali extractions would release 5.0% of TOC_s. For the sites with minimal reductive dechlorination (Figure 6-5B), a lower number of samples contributed to the trends but little impact was seen in the correlation coefficients between the sites with active or minimal reductive dechlorination activity. The

regressions for the pyrophosphate and alkali extracts intersected in Figure 6-5b which indicated that sediment with high TOC_s produced more alkali extracted organic carbon than pyrophosphate extracted organic carbon for the minimal reductive dechlorination sites. A comparison between the regressions in Figure 6-5 to the regression in Figure 6-2 showed that the pyrophosphate extractions for the active reductive dechlorination sites dominated in the PBOC trend. Overall, the pyrophosphate extractions removed more organic carbon in the sites with active reductive dechlorination while the trend in the sites with minimal reductive dechlorination depended on the quantity of TOC_s. For this data, the pyrophosphate extractions indicated a better measure to assess the readily available organic carbon because the sites exhibiting reductive dechlorination contained more pyrophosphate extracts.

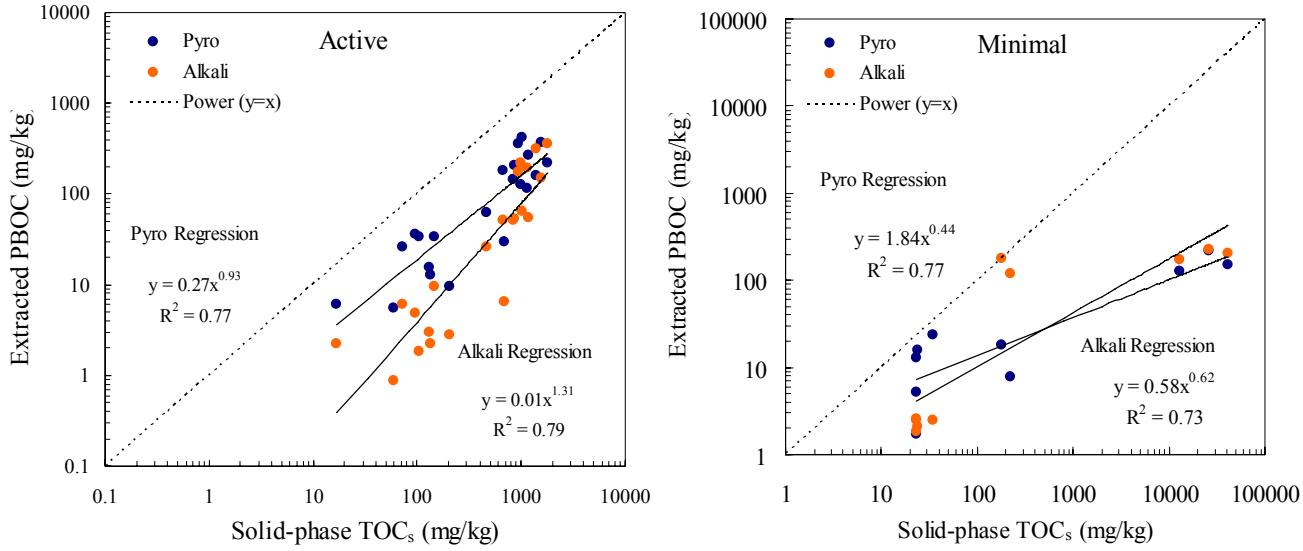

Figure 6-5. TOC_{aq} (mg/kg) versus the sediment total organic carbon (TOC_s, mg/kg) for the six study sites where graph (A) included sites with active reductive dechlorination and graph (B) included sites with minimal reductive dechlorination.

Bioassay Experiments

Bioassays were run on the pyrophosphate and alkali extractions for the sediments to test the biodegradability of the extracted organic carbon. Again, the results were divided between the sites with active reductive dechlorination and minimal reductive dechlorination. In Figure 6-6, the decrease in aqueous TOC (ΔTOC_{aq}) in the bioassays was plotted against the extracted organic carbon concentration. The ΔTOC_{aq} represented the maximum utilization in TOC_{aq} over 45 days. For the sites with active reductive dechlorination, the alkali extraction showed that 30% of the extracted organic carbon was utilized whereas the pyrophosphate extraction bioassays

exhibited a 19% utilization of TOC_{aq} assuming an initial extracted TOC of 10 mg/L. Although the pyrophosphate extractions removed more carbon, the pyrophosphate extractions exhibited less biodegradation. The bioassay results from sites with minimal reductive dechlorination activity also demonstrated (Figure 6-7) that the alkali extractions contained more biodegradable organic carbon than the pyrophosphate extractions. However, it is possible that the nature of the extracted organic carbon was altered by hydrolysis during the base extractions (Stevenson 1994). As a result, these alterations may cause the extracted carbon to be more biodegradable. Therefore, the direct comparison of the biodegradability for the pyrophosphate and base extracts might not be appropriate. Nevertheless, the utilization of the TOC_{aq} in both the pyrophosphate and base extracts showed that the aquifer sediments contained readily biodegradable organic carbon which could contribute to the reductive dechlorination observed at the sites.

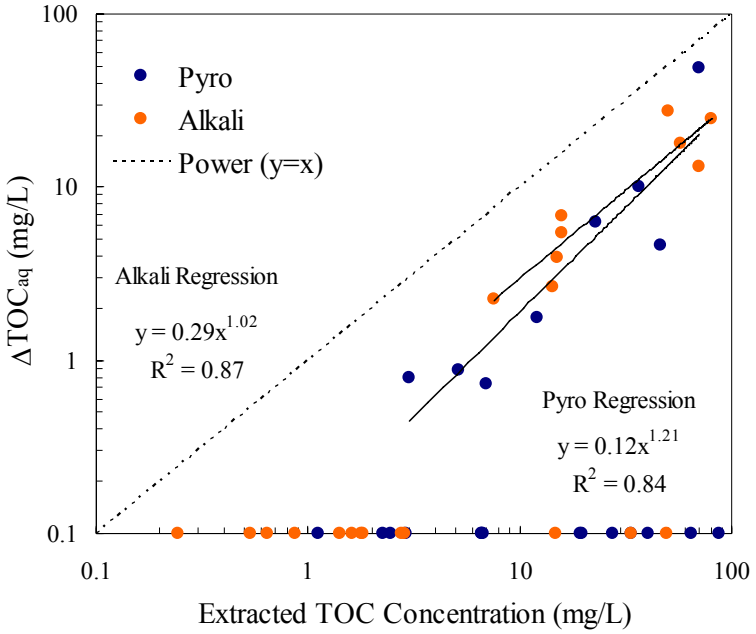

Figure 6-6. Summary of bioassays for sites with active reductive dechlorination where the delta ΔTOC_{aq} (mg/L) was the maximum utilization of aqueous TOC over 45 days in the bioassays and the extracted TOC concentrations were the initial TOC concentrations in the bioassays. Due to the use of the log-log scale, all bioassays that exhibited no utilization of TOC_{aq} are shown on the x-axis at an artificial value of ΔTOC_{aq}=0.1 mg/L.

As with the PBOC extractions, the data set for the sites with minimal reductive dechlorination was smaller in comparison to the sites with active reductive dechlorination. This reduction in data points was increased further due to the number of bioassays displaying no biodegradation. For the sites with minimal reductive dechlorination, all extractions below the

TOC threshold of 3 mg/L (as noted in the Chapter 5) exhibited no biodegradation. When the TOC_{aq} was above 3 mg/L, all extractions at the sites with minimal reductive dechlorination demonstrated biodegradability. However, only 39% of the sites with active reductive dechlorination showed biodegradation in the bioassays. Although 14 of the extractions were also below 3mg/L for the active reductive dechlorination sites, 10 extractions were above 3 mg/L and still did not exhibit a decrease in TOC over 45 days. This variability in the biodegradability of the PBOC extraction was assumed to be a result of the reductive dechlorination activity at the sites. As demonstrated in Chapter 5, the PBOC extractions inside the plume exhibited reduced biodegradability in comparison to the PBOC extractions outside of the plume. The presumed reason for the reduced biodegradability is a result of the microbial activity supporting and conducting reductive dechlorination in the aquifer.

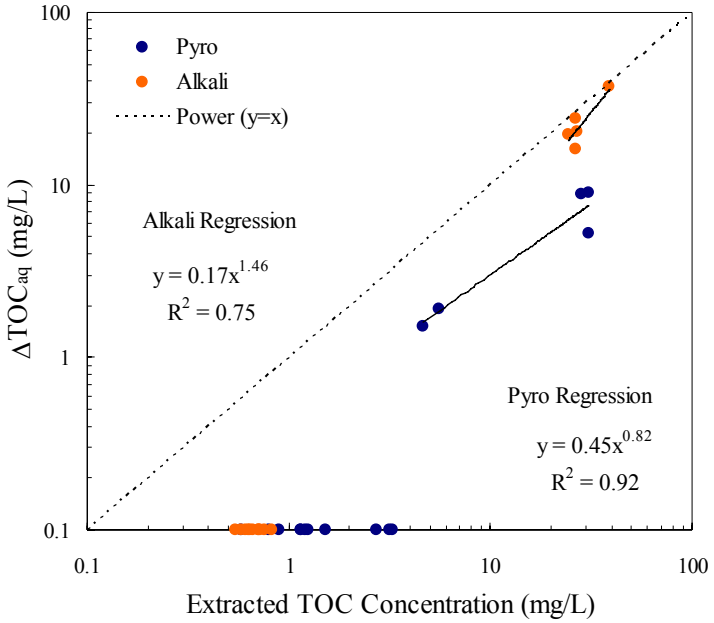

Figure 6-7. Summary of bioassays for sites with minimal reductive dechlorination where the ΔTOC_{aq} (mg/L) was the maximum utilization of aqueous TOC over 45 days in the bioassays and the extracted TOC concentrations were the initial TOC concentrations in the bioassays. Due to the use of the log-log scale, all bioassays that exhibited no utilization of TOC_{aq} are shown on the x-axis at an artificial value of ΔTOC_{aq}=0.1 mg/L.

Conclusions

In this study, the distribution of the PBOC extractions was investigated across six contaminated sites that encompassed a wide range of organic carbon content and reductive

dechlorination activity. PBOC extractions were directly correlated quantitatively to TOC_s and soft carbon content and qualitatively to the level of reductive dechlorination activity at the sites. The correlations supported the assumption that the soft carbon content serves as the upper bound of bioavailable organic carbon associated with the sediment. When PBOC was separated into the pyrophosphate and alkali PBOC extracts, the correlations showed that the pyrophosphate extractions removed more organic carbon than the alkali extractions for the active reductive dechlorination sites. Both the pyrophosphate and alkali extracts contained biodegradable organic carbon at the active and minimal reductive dechlorination sites. Based on the empirical correlations and bioassay results, a range for bioavailable organic carbon associated with the sediment was proposed where the upper bound consisted of the soft carbon and the lower bound consisted of the PBOC. At the sites with active reductive dechlorination, the biodegradability of the PBOC extracts was lower which indicated an impact on the sediment-associated organic carbon due to reductive dechlorination. Overall, the variability in the PBOC extractions correlated well with reductive dechlorination activity at a site.

References

Butler, E. C. and K. F. Hayes (1999). "Kinetics of the transformation of trichloroethylene and tetrachloroethylene by iron sulfide." Environmental Science & Technology **33**(12): 2021-2027.

Chapelle, F. H. and P. M. Bradley (1998). "Selecting Remediation Goals by Assessing the Natural Attenuation Capacity of Groundwater Systems." Journal Bioremediation **2**: 227-238.

Chapelle, F. H., P. M. Bradley and C. C. Casey (2005). "Behavior of a chlorinated ethene plume following source-area treatment with Fenton's reagent." Ground Water Monitoring and Remediation **25**(2): 131-141.

Chapelle, F. H., J. T. Novak, J. Parker, B. B. Campbell and M. A. Widdowson (2006). A Framework for Assessing the Sustainability of Monitored Natural Attenuation USGS Circular Series C-1303., United States Geological Survey.

Chapelle, F. H., M. A. Widdowson, J. S. Brauner, E. Mendez and C. C. Casey (2003). Methodology for Estimating Times of Remediation Associated with Monitored Natural Attenuation. WRIR 03-4057, USGS.

Cuypers, C., T. Grotenhuis, J. Joziasse and W. Rulkens (2000). "Rapid persulfate oxidation predicts PAH bioavailability in soils and sediments." Environmental Science & Technology **34**(10): 2057-2063.

EPA (1999). Final OSWER monitored natural attenuation policy. Directive 9200.4-17. O. o. S. W. a. E. Response, United States Environmental Protection Agency.

Fetter, C. W. (1999). Contaminant Hydrogeology. Upper Saddle River, NJ, Prentice Hall.

Maymo-Gatell, X., Y. T. Chien, J. M. Gossett and S. H. Zinder (1997). "Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene." Science **276**(5318): 1568-1571.

Mohn, W. W. and J. M. Tiedje (1992). "Microbial Reductive Dehalogenation." Microbiological Reviews **56**(3): 482-507.

Wiedemeier, T. H., M. A. Swanson, D. E. Moutoux, E. K. Gorden, J. T. Wilson, B. H. Wilson, D. H. Kambell, P. Haas, R. N. Miller, J. E. Hansen and F. H. Chapelle (1998). Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water. Washington, DC, U.S. EPA.

Chapter 7. Engineering Significance

The results of this research provide engineers and scientists with simple laboratory methods to estimate the range of the bioavailable organic carbon associated with the aquifer sediment. The proposed upper bound of the organic carbon is estimated by the soft carbon content of the aquifer sediment. The proposed lower bound of the organic carbon is estimated by the potentially bioavailable organic carbon (PBOC) extraction method. In this study the PBOC method has been shown to be relevant to long-term reductive dechlorination at a site. However, the ability to assess the sustainability of reductive dechlorination and also the sustainability of monitored natural attenuation (MNA) has not been fully explored.

The sustainability of reductive dechlorination depends on the supply of reducing compounds. The supply of metabolizable organic carbon available in a ground-water system can be estimated by the PBOC method. Therefore, based on the range of the bioavailable organic carbon associated with the aquifer sediment generated by the PBOC method, an estimate of the degradable PCE (g) can also be calculated. If the mass of PCE contamination in the aquifer is known, then the estimated range of degradable PCE using the PBOC values can be compared to the known contaminant mass. From this comparison, the potential of the aquifer to completely degrade a known contaminant mass can be assessed.

To estimate the range of degradable PCE, the following calculation can be used. For a cubic meter of aquifer sediment assuming a bulk density of 2.35 g/cm^3, the total quantity of sediment mass is 2350 kg. Based on the empirical expressions and the TOC_s (mg/kg) at a site, the range of bioavailable organic carbon (mg) can be calculated for the cubic volume of aquifer sediment. Assuming that aquifer sediment TOC_s is 100 mg/kg, the empirical expressions predict 30-51 mg/kg bioavailable organic carbon which translates to 71-120 g of bioavailable organic carbon per cubic volume. The conversion of bioavailable organic carbon to mass of PCE degraded depends on the following assumptions. First, to degrade PCE through VC, 21 mg PCE requires 1 mg H_2 (Wiedemeier et al. 1999). Second, H_2 has 1 reducing equivalent per g (Fennell et al.

1997). Third to connect organic carbon to reducing equivalents, it was assumed that 1 mg carbon contains 2 reducing equivalents (a conservative assumption based on the reducing equivalents supplied by yeast extract (Fennell et al. 1997)). Finally, Wiedemeier et al. (1999) recommends assuming that 25 to 100 times the required organic carbon be added due to competition for the H_2 by microbes other than dechlorinators. From these assumptions, the range of PCE that can be degraded is 29-200 g per cubic volume of aquifer matrix. The calculation indicates the reductive capacity of an aquifer for a given range of PBOC and a known mass of contaminant to support reductive dechlorination. However, the release rate of PBOC from the aquifer sediment and the dissolution rate of the chloroethene into the ground water have not been addressed.

To assess the time of remediation for chloroethene sites, either the dissolution of the chloroethene or the release of PBOC will be the rate limiting step. If all the PBOC is considered readily available, dissolution of the chloroethene could be responsible for the quantity of biodegradable contaminant and control the utilization rate of the PBOC. If PBOC is released slowly into the ground water, biodegradation of the contaminants is limited by the PBOC release rate. However, the release mechanism for PBOC is not known. Thus, the release rate of PBOC into the ground water is also not known. Therefore to assess the time scale of remediation, a predictive model should be incorporated in the evaluation of reductive dechlorination sustainability to address the relationship between the availability of organic carbon as a driving mechanism for reductive dechlorination and the effect of dissolution rate on quantity and rate of chloroethene released into the aqueous phase.

To enable a predictive assessment of reductive dechlorination sustainability, the next step for this research is development of a mathematical model to integrate the range of bioavailable organic carbon associated with the aquifer sediment into Sequential Electron Acceptor Model, 3 Dimensional (SEAM3D). Chapelle et al. (2006) demonstrated this approach at a chloroethene-contaminated site using SEAM3D to find a solution for electron donor/acceptor mass and energy balance in a ground water. In this study, PBOC was instrumental in quantifying the organic carbon flux into the chloroethene plume but there was no other source term in the model for the aquifer sediment. With the use of SEAM3D, the PBOC can be incorporated into a predictive model where all the

contributing factors of MNA including reductive dechlorination can be included to address the sustainability of MNA at contaminated sites. Aspects of MNA remediation strategies, such as time of remediation and source zone treatment, could be evaluated with the added component of bioavailable organic carbon to SEAM3D. The long-term goal of the research is to provide engineers with laboratory methods as well as a predictive model to employ at sites contaminated with chlorinated solvents for the assessment of the sustainability of MNA as a remediation strategy.

References

Chapelle, F. H., J. T. Novak, J. Parker, B. B. Campbell and M. A. Widdowson (2006). A Framework for Assessing the Sustainability of Monitored Natural Attenuation USGS Circular Series C-1303., United States Geological Survey.

Fennell, D. E., J. M. Gossett and S. H. Zinder (1997). "Comparison of butyric kid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of tetrachloroethene." Environmental Science & Technology **31**(3): 918-926.

Wiedemeier, T. H., H. S. Rifai, C. J. Newell and J. T. Wilson (1999). Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface. New York, John Wiley & Sons, Inc.

Appendix A. Chapter 3

Table 3-1. Total Organic Carbon (TOC_s mg C/kg sediment), Soft Carbon (mg/kg), and Extracted Aqueous Carbon Concentrations (TOC_{aq} mg/kg) for parallel Nanopure™ water, 0.1% pyrophosphate, 0.5% pyrophosphate, 1.0% pyrophosphate, and base extractions for SWS-Edisto, SWS-Stroubles, AQU-Columbia Deep, and AQU-Columbia Shallow sediment. The values for the extracted TOC_{aq} are shown with standard deviations of triplicate or quadruplicate samples.

Table 3-1 data for SWS-Edisto Sediment

Replicates	Total Organic Carbon (mg/kg)	Soft Carbon (mg/kg)	Extracted Aqueous Carbon Concentration (mg/kg)				
			Nanopure	0.1% Pyro	0.5% Pyro	1.0% Pyro	Base
1	14269	6318	144	210	776	986	2770
2	13879	7182	117	170	762	1120	2516
3	10986	3805	114	251	725	1093	2587
Average	13045	5770	125	210	754	1070	2620
Standard Deviation	1794	1754	17	40	26	71	131

Table 3-1 data for SWS-Stroubles Sediment

Replicates	Total Organic Carbon (mg/kg)	Soft Carbon (mg/kg)	Extracted Aqueous Carbon Concentration (mg/kg)				
			Nanopure	0.1% Pyro	0.5% Pyro	1.0% Pyro	Base
1	9840	5038	108.44	157.60	1633.40	2319.12	1032.99
2	14709	7531	151.28	150.74	1316.90	1742.67	945.87
3	11689	5985	71.01	126.32	1488.90	1907.19	1371.23
4			102.43	122.73	1565.67	1837.47	1116.70
5			79.74	121.29	1038.60	1491.48	1047.73
6			79.46				
Average	12079	6185	99	136	1409	1860	1100
Standard Deviation	2458	1258	30	17	238	301	162

Table 3-1 data for Deep AQU-Columbia Sediment

Replicates	Total Organic Carbon (mg/kg)	Soft Carbon (mg/kg)	Extracted Aqueous Carbon Concentration (mg/kg)				
			Nanopure	0.1% Pyro	0.5% Pyro	1.0% Pyro	Base
1	1214	742	15.2	19.0	30.7	51.8	125.5
2	1235	754	16.7	21.2	30.1	45.9	121.9
3	1106	676	18.7	20.2	30.2	49.0	124.7
Average	1185	724	16.9	20.1	30.4	48.9	124.0
Standard Deviation	69	42	1.8	1.1	0.3	3.0	1.9

Table 3-1 data for Shallow AQU-Columbia Sediment

Replicates	Total Organic Carbon (mg/kg)	Soft Carbon (mg/kg)	Extracted Aqueous Carbon Concentration (mg/kg)				
			Nanopure	0.1% Pyro	0.5% Pyro	1.0% Pyro	Base
1	239	190	5.0	23.4	20.5	27.1	54.6
2	136	108	4.7	23.2	14.9	25.8	54.3
3	187	149	3.8	24.1	22.3	25.4	52.8
Average	187.3	148.9	4.5	23.6	19.2	26.1	53.9
Standard Deviation	51.9	41.2	0.6	0.5	3.8	0.9	1.0

Figure 3-2. A) Decrease in TOC_{aq} concentration (solid lines) over time and B) increase in cumulative CO_2 (dotted lines) over time for Nanopure™ water, 0.1% pyrophosphate, 0.5% pyrophosphate, 1.0% pyrophosphate, base, and control base extractions of SWS-Stroubles sediment.

Figure 3-2 A: Aqueous TOC (mg/L) Data

Time	Nanopure	StdDev	0.1% pyro	Std Dev	0.5% pyro	Std Dev	1.0% pyro	Std dev	Base	Std dev	Control	Std dev
0	82.20	3.30	78.94	3.76	242.60	16.48	255.15	11.16	155.63	8.17	133.47	3.47
24	71.55	2.58	82.01	6.57	226.51	9.81	229.32	16.79	153.93	5.64	123.02	20.41
43	50.06	0.96	57.05	4.73	188.70	9.88	199.70	7.48	94.20	8.54	125.49	15.50

Figure 3-2 B: Cumulative CO2-C (%) Data

Time	Nanopure	StdDev	0.1% pyro	Std Dev	0.5% pyro	Std Dev	1.0% pyro	Std dev	Base	Std dev	Control	Std dev
0	0	0	0	0	0	0	0	0	0	0	0	0
24	7.44	2.11	7.69	2.71	4.07	0.69	4.33	0.66	15.24	0.53	0.03	0.04
43	9.38	0.27	10.16	0.36	5.64	0.48	5.56	0.36	20.46	0.70	0.33	0.26

Figure 3-3. Percent of extracted TOC_{aq} relative to the TOC_s (plain bars) and percent of extracted TOC_{aq} utilized in the bioassays relative to the TOC_s (hatched bars) for Nanopure™ water, 0.1% pyrophosphate, 0.5% pyrophosphate, 1.0% pyrophosphate, and base extractions of SWS-Stroubles sediment.

Figure 3-3: Summary Data

Extractant	Extraction Efficiency (%, TOC_{aq}/TOC_s)	Std Dev	TOC Utilization (%, $\Delta TOC_{aq}/TOC_s$)	Std Dev
Nano	0.51	0.01	0.20	0.00
0.1% Pyro	0.90	0.02	0.25	0.01
0.5% Pyro	11.03	0.39	2.45	0.11
1.0% Pyro	13.52	0.46	2.94	0.09
Base	9.90	0.08	3.91	0.17

Figure 3-4. Quantity of extracted TOC_{aq} (mg/kg) for a two-step extraction sequence on SWS-Stroubles sediment where the standard deviations of triplicate extractions are shown with error bars. The initial extractions were run in parallel and used Nanopure water, 0.1% pyrophosphate, 0.5% pyrophosphate, or 1.0% pyrophosphate as an extracting solution. The second extraction run in sequence used 0.5 N sodium hydroxide (base) as an extracting solution in all the samples.

Figure 3-4: Double extraction

Extractant	TOC_{aq} (mg/kg)	Std Dev	Extractant Sequence	TOC_{aq} (mg/kg)	Std Dev
Nano	98.73	29.56	Nano-Base	1018.69	40.83
0.1% Pyro	135.74	17.10	0.1 Pyro-Base	1281.32	155.91
0.5% Pyro	1408.69	238.21	0.5 Pyro-Base	1329.10	32.49
1.0% Pyro	1859.58	301.29	1.0 Pyro-Base	1339.56	44.14

Figure 3-5. Cumulative extraction efficiencies from repetitive (solid line) and alternating (dotted lines) extractions of SWS-Stroubles sediment. Repetitive extractions used the same extractant over 8 extractions, and the 0.1% pyro alternating extraction alternated between 0.1% pyro (E1, E3, E5, E7) and base (E2, E4, E6, E8) extractants.

Figure 3-5: SWS-Stroubles Multiple Extraction Experiment

Extraction Number	0.1% Pryo Cumulative Extraction Eff (%)	Base Cumulative Extraction Eff (%)	Alternating Cumulative Extraction Eff (%)
1	0.89	9.90	0.91
2	4.05	12.23	4.97
3	6.58	13.82	11.19
4	9.17	15.04	12.66
5	10.33	15.40	14.15
6	13.66	21.92	17.20
7	15.60	24.79	19.09
8	17.04	26.51	21.17

Figure 3-6. Cumulative extraction efficiency from repetitive (solid line) and alternating (dotted lines) extractions using AQU-Columbia sediment from shallow (A) and deep (B) depths. Repetitive extractions used the same extractant over 8 extractions, and the 0.1% pyro alternating extraction alternated between 0.1% pyro (E1, E3, E5, E7) and base (E2, E4, E6, E8) extractants.

Figure 3-6A: Shallow AQU-Columbia Multiple Extraction Experiment

Extraction Number	0.1% Pryo Cumulative Extraction Eff (%)	Base Cumulative Extraction Eff (%)	Alternating Cumulative Extraction Eff (%)	0.1% Pyro Std Dev	Base Std Dev	Alternating Std Dev
1	33.24	60.81	31.26	1.58	1.14	2.53
2	47.67	74.43	66.83	0.77	2.56	4.31
3	57.92	81.45	77.47	1.43	2.72	3.05
4	65.72	90.39	86.66	1.78	3.14	3.99
6	80.25	102.44	98.88	3.11	2.76	0.43
8	91.00	112.67	109.16	3.33	2.33	0.95
10	94.37	115.83		3.36	2.24	
12	96.91	118.05		3.32	2.37	
14	101.33	122.69		3.12	2.60	
16	108.29	127.65		2.02	2.31	
18	113.17	132.49		1.88	2.54	
20	118.91	137.90		1.83	2.05	

Figure 3-6B: Deep AQU-Columbia Multiple Extraction Experiment

Extraction Number	0.1% Pryo Cumulative Extraction Eff (%)	Base Cumulative Extraction Eff (%)	Alternating Cumulative Extraction Eff (%)	0.1% Pyro Std Dev	Base Std Dev	Alternating Std Dev
1	30.02	114.11	30.15	1.95	0.78	2.46
2	55.08	149.34	151.31	0.55	1.62	1.34
3	76.31	168.21	210.84	0.74	1.37	14.78
4	93.59	188.97	229.26	1.94	3.03	14.35
6	104.49	207.77	245.47	1.25	3.20	9.12
8	118.16	231.31	268.63	0.68	2.98	10.14
10	123.69	241.38		1.17	0.74	
12	129.87	246.84		1.34	0.82	
14	136.29	254.01		1.15	1.02	
16	141.91	260.50		2.07	2.80	
18	148.40	267.54		2.31	3.26	
20	153.66	274.27		1.31	1.74	

Table 3-2. Linear and rectangular hyperbola regression fit data for multiple extractions using either 0.1% pyrophosphate or base solution on SWS-Stroubles sediment regression and shallow and deep AQU-Columbia sediments, respectively.

SWS-Stroubles 0.1% Pyrophosphate Regression

Nonlinear Regression

Data Source: Data 6 in MethodsPaper.JNB
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9989	0.9978	0.9971	0.5940

	Coefficient	Std. Error	t	P	VIF
y0	-0.6878	0.4628	-1.4862	0.1878	4.8571<
a	2.3006	0.0917	25.1011	<0.0001	4.8571<

Analysis of Variance:

	DF	SS	MS
Regression	2	969.5400	484.7700
Residual	6	2.1168	0.3528
Total	8	971.6568	121.4571

Statistical Tests:

PRESS	4.4843	
Durbin-Watson Statistic	1.9265	Passed
Normality Test	Passed	(P = 0.7567)

K-S Statistic = 0.2251 Significance Level = 0.7567

Constant Variance Test	Passed	(P = 0.9309)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	1.6127	-0.7265	-1.2230	-1.6013	-1.9318
2	3.9133	0.1394	0.2347	0.2754	0.2530
3	6.2139	0.3625	0.6104	0.6735	0.6394
4	8.5144	0.6520	1.0976	1.1774	1.2257
5	10.8150	-0.4806	-0.8092	-0.8680	-0.8474
6	13.1155	0.5438	0.9156	1.0102	1.0123
7	15.4161	0.1825	0.3073	0.3606	0.3328
8	17.7167	-0.6731	-1.1333	-1.4838	-1.7024

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.9158	0.4167	-1.6327
2	0.0143	0.2738	0.1554
3	0.0493	0.1786	0.2981
4	0.1044	0.1310	0.4758
5	0.0568	0.1310	-0.3289
6	0.1109	0.1786	0.4720
7	0.0245	0.2738	0.2043
8	0.7863	0.4167	-1.4388

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	1.6127	0.6746	2.5509	-0.1172	3.3426
2	3.9133	3.1528	4.6738	2.2729	5.5536

3	6.2139	5.5997	6.8280	4.6360	7.7917
4	8.5144	7.9885	9.0404	6.9688	10.0601
5	10.8150	10.2890	11.3409	9.2693	12.3606
6	13.1155	12.5014	13.7297	11.5377	14.6934
7	15.4161	14.6556	16.1766	13.7758	17.0565
8	17.7167	16.7785	18.6548	15.9868	19.4466

Fit Equation Description:

[Variables]
x = col(4)
y = col(1)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=ape(x,y,1,0,1)
[Parameters]
y0 = F(0)[1] "Auto {{previous: -0.687836}}
a = F(0)[2] "Auto {{previous: 2.30056}}
[Equation]
f=y0+a*x
fit f to y
"fit f to y with weight reciprocal_ysquare
"fit f to y with weight reciprocal_y
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 1

SWS-Stroubles Base Regression

Nonlinear Regression

Data Source: Data 6 in MethodsPaper.JNB
Equation: Linear

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9970	0.9939	0.9919	1.6511

	Coefficient	Std. Error	t	P	VIF
y0	6.5401	1.2865	5.0836	0.0023	4.8571<
a	2.4245	0.2548	9.5166	<0.0001	4.8571<

Analysis of Variance:

	DF	SS	MS
Regression	2	2683.0835	1341.5418
Residual	6	16.3567	2.7261
Total	8	2699.4402	337.4300

Statistical Tests:

PRESS 25.0519

Durbin-Watson Statistic 1.4634 Failed

Normality Test Passed (P = 0.5324)

K-S Statistic = 0.2702 Significance Level = 0.5324

Constant Variance Test Passed (P = 0.9309)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	8.9647	0.9370	0.5675	0.7430	0.7118
2	11.3892	0.8457	0.5122	0.6011	0.5660
3	13.8138	0.0058	0.0035	0.0039	0.0036
4	16.2383	-1.2008	-0.7273	-0.7802	-0.7513
5	18.6629	-3.2668	-1.9786	-2.1224<	-3.8810<
6	21.0874	0.8315	0.5036	0.5557	0.5208
7	23.5120	1.2782	0.7741	0.9084	0.8930
8	25.9365	0.5694	0.3449	0.4515	0.4194

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.1972	0.4167	0.6016
2	0.0681	0.2738	0.3476
3	1.6511E-006	0.1786	0.0017
4	0.0459	0.1310	-0.2916
5	0.3394	0.1310	-1.5065
6	0.0336	0.1786	0.2428
7	0.1556	0.2738	0.5483
8	0.0728	0.4167	0.3544

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	8.9647	6.3568	11.5725	4.1560	13.7733
2	11.3892	9.2752	13.5033	6.8295	15.9490
3	13.8138	12.1065	15.5210	9.4278	18.1998
4	16.2383	14.7763	17.7003	11.9419	20.5348
5	18.6629	17.2009	20.1249	14.3664	22.9594
6	21.0874	19.3802	22.7947	16.7014	25.4734

7	23.5120	21.3979	25.6260	18.9522	28.0717
8	25.9365	23.3287	28.5444	21.1279	30.7452

Fit Equation Description:

[Variables]
x = col(4)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=ape(x,y,1,0,1)
[Parameters]
y0 = F(0)[1] "Auto {{previous: 6.54013}}
a = F(0)[2] "Auto {{previous: 2.42455}}
[Equation]
f=y0+a*x
fit f to y
"fit f to y with weight reciprocal_ysquare
"fit f to y with weight reciprocal_y
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 1

AQU-Columbia Shallow 0.1% Pyrophosphate Regression

Nonlinear Regression

Data Source: Data 12 in MethodsPaper.JNB
Equation: Single Rectangular, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9992	0.9984	0.9983	1.7798

	Coefficient	Std. Error	t	P	VIF
a	59.1780	1.3228	44.7382	<0.0001	5.7799<
b	3.1921	0.2782	11.4756	<0.0001	5.7799<

Analysis of Variance:

	DF	SS	MS
Regression	2	36646.3565	18323.1782
Residual	18	57.0193	3.1677
Total	20	36703.3758	1835.1688

Statistical Tests:

PRESS 72.8174

Durbin-Watson Statistic 1.0085 Failed

Normality Test Passed (P = 0.9837)

K-S Statistic = 0.0998 Significance Level = 0.9837

Constant Variance Test Passed (P = 0.7918)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	14.1167	3.6139	2.0305	2.1879<	2.4817<
2	22.7956	1.7373	0.9761	1.0883	1.0942
3	28.6712	0.1269	0.0713	0.0787	0.0765
4	32.9130	-0.9355	-0.5256	-0.5683	-0.5574
5	36.1191	-4.1417	-2.3270	-2.4695<	-2.9515<
6	38.6277	0.4174	0.2345	0.2455	0.2389
7	40.6440	-1.0605	-0.5959	-0.6180	-0.6071
8	42.3000	1.8762	1.0541	1.0875	1.0934
9	43.6843	0.7959	0.4472	0.4602	0.4499
10	44.8588	-0.3273	-0.1839	-0.1892	-0.1840
11	45.8677	-0.9535	-0.5357	-0.5519	-0.5410
12	46.7439	-1.7938	-1.0078	-1.0409	-1.0434
13	47.5118	-1.4594	-0.8200	-0.8497	-0.8428
14	48.1904	-1.2518	-0.7033	-0.7317	-0.7219
15	48.7943	-0.6308	-0.3544	-0.3703	-0.3613
16	49.3354	0.6816	0.3830	0.4020	0.3924
17	49.8228	0.6394	0.3592	0.3790	0.3698
18	50.2643	1.3580	0.7630	0.8089	0.8008
19	50.6659	0.9563	0.5373	0.5725	0.5615
20	51.0330	2.3415	1.3156	1.4090	1.4516

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.3855	0.1387	0.9960
2	0.1439	0.1955	0.5394
3	0.0007	0.1794	0.0358
4	0.0273	0.1447	-0.2292
5	0.3849	0.1121	-1.0487
6	0.0029	0.0872	0.0739
7	0.0145	0.0704	-0.1670

8	0.0380	0.0604	0.2772
9	0.0063	0.0558	0.1094
10	0.0010	0.0553	-0.0445
11	0.0093	0.0578	-0.1339
12	0.0361	0.0624	-0.2693
13	0.0266	0.0687	-0.2288
14	0.0220	0.0760	-0.2070
15	0.0063	0.0840	-0.1094
16	0.0082	0.0926	0.1253
17	0.0081	0.1014	0.1242
18	0.0406	0.1103	0.2820
19	0.0222	0.1193	0.2066
20	0.1459	0.1282	0.5566

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	14.1167	12.7240	15.5094	10.1265	18.1069
2	22.7956	21.1422	24.4489	18.7071	26.8841
3	28.6712	27.0874	30.2551	24.6104	32.7321
4	32.9130	31.4906	34.3353	28.9123	36.9136
5	36.1191	34.8672	37.3710	32.1759	40.0624
6	38.6277	37.5235	39.7319	34.7288	42.5266
7	40.6440	39.6520	41.6359	36.7754	44.5126
8	42.3000	41.3810	43.2189	38.4495	46.1505
9	43.6843	42.8010	44.5676	39.8422	47.5265
10	44.8588	43.9796	45.7379	41.0176	48.7000
11	45.8677	44.9691	46.7664	42.0220	49.7135
12	46.7439	45.8096	47.6782	42.8897	50.5981
13	47.5118	46.5320	48.4916	43.6463	51.3773
14	48.1904	47.1597	49.2211	44.3117	52.0691
15	48.7943	47.7104	49.8783	44.9011	52.6875
16	49.3354	48.1977	50.4730	45.4269	53.2439
17	49.8228	48.6323	51.0133	45.8986	53.7470
18	50.2643	49.0224	51.5061	46.3242	54.2043
19	50.6659	49.3746	51.9572	46.7100	54.6219
20	51.0330	49.6943	52.3716	47.0613	55.0046

Fit Equation Description:

[Variables]
x = col(37)
y = col(38)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 59.178}}
b = x50(x,y,0.1) "Auto {{previous: 3.19205}}
[Equation]
f=a*x/(b+x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Options]
tolerance=1e-10
stepsize=1
iterations=200
[Constraints]

Number of Iterations Performed = 9

AQU-Columbia Shallow Base Regression

Nonlinear Regression

Data Source: Data 12 in MethodsPaper.JNB
Equation: Single Rectangular, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9990	0.9979	0.9977	2.6937

	Coefficient	Std. Error	t	P	VIF
a	67.3136	1.3361	50.3808	<0.0001	3.4011
b	1.5048	0.1754	8.5767	<0.0001	3.4011

Analysis of Variance:

	DF	SS	MS
Regression	2	62640.7757	31320.3879
Residual	18	130.6119	7.2562
Total	20	62771.3877	3138.5694

Statistical Tests:

PRESS 198.5165

Durbin-Watson Statistic 0.6208 Failed

Normality Test Passed (P = 0.8901)

K-S Statistic = 0.1255 Significance Level = 0.8901

Constant Variance Test Passed (P = 0.5001)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	26.8740	6.9360	2.5749<	3.0666<	4.3127<
2	38.4124	1.6886	0.6269	0.7239	0.7140
3	44.8281	-2.2445	-0.8332	-0.9143	-0.9099
4	48.9128	-2.1173	-0.7860	-0.8352	-0.8279
5	51.7416	-4.9461	-1.8361	-1.9162	-2.0873<
6	53.8166	-1.3261	-0.4923	-0.5089	-0.4981
7	55.4036	-2.4431	-0.9070	-0.9333	-0.9298
8	56.6566	0.4203	0.1560	0.1603	0.1559
9	57.6711	-0.4095	-0.1520	-0.1562	-0.1519
10	58.5093	-1.1638	-0.4320	-0.4446	-0.4344
11	59.2133	-0.9887	-0.3670	-0.3784	-0.3692
12	59.8132	-1.5885	-0.5897	-0.6095	-0.5985
13	60.3303	-1.1538	-0.4283	-0.4438	-0.4337
14	60.7807	-0.7120	-0.2643	-0.2746	-0.2674
15	61.1765	0.5999	0.2227	0.2320	0.2258
16	61.5271	1.1389	0.4228	0.4416	0.4315
17	61.8398	1.5499	0.5754	0.6026	0.5916
18	62.1204	2.6087	0.9684	1.0169	1.0179
19	62.3737	2.3554	0.8744	0.9204	0.9163
20	62.6034	3.8065	1.4131	1.4910	1.5477

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	1.9676	0.2950	2.7898<
2	0.0874	0.2502	0.4124
3	0.0853	0.1694	-0.4110
4	0.0450	0.1143	-0.2974
5	0.1637	0.0818	-0.6232
6	0.0089	0.0642	-0.1304
7	0.0256	0.0556	-0.2256

8	0.0007	0.0526	0.0368
9	0.0007	0.0531	-0.0360
10	0.0058	0.0556	-0.1054
11	0.0045	0.0594	-0.0928
12	0.0127	0.0638	-0.1563
13	0.0073	0.0686	-0.1177
14	0.0030	0.0736	-0.0754
15	0.0023	0.0786	0.0660
16	0.0089	0.0835	0.1303
17	0.0176	0.0883	0.1842
18	0.0530	0.0930	0.3259
19	0.0457	0.0975	0.3011
20	0.1259	0.1018	0.5209

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	26.8740	23.8002	29.9479	20.4338	33.3143
2	38.4124	35.5819	41.2430	32.0847	44.7402
3	44.8281	42.4986	47.1576	38.7081	50.9481
4	48.9128	46.9994	50.8263	42.9388	54.8869
5	51.7416	50.1226	53.3607	45.8553	57.6280
6	53.8166	52.3831	55.2501	47.9785	59.6546
7	55.4036	54.0690	56.7381	49.5890	61.2181
8	56.6566	55.3581	57.9552	50.8502	62.4630
9	57.6711	56.3671	58.9752	51.8635	63.4788
10	58.5093	57.1747	59.8438	52.6947	64.3238
11	59.2133	57.8345	60.5922	53.3885	65.0382
12	59.8132	58.3836	61.2427	53.9760	65.6503
13	60.3303	58.8476	61.8129	54.4799	66.1806
14	60.7807	59.2452	62.3161	54.9167	66.6446
15	61.1765	59.5898	62.7632	55.2989	67.0540
16	61.5271	59.8914	63.1628	55.6361	67.4180
17	61.8398	60.1577	63.5219	55.9358	67.7438
18	62.1204	60.3947	63.8462	56.2038	68.0370
19	62.3737	60.6069	64.1405	56.4450	68.3024
20	62.6034	60.7981	64.4087	56.6631	68.5437

Fit Equation Description:

[Variables]
x = col(37)
y = col(39)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 67.3136}}
b = x50(x,y,0.1) "Auto {{previous: 1.50478}}
[Equation]
f=a*x/(b+x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Options]
tolerance=1e-10
stepsize=1
iterations=200
[Constraints]

Number of Iterations Performed = 10

AQU-Columbia Deep 0.1% Pyrophosphate Regression

Nonlinear Regression

Data Source: Data 14 in MethodsPaper.JNB
Equation: Single Rectangular, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9997	0.9994	0.9994	0.2617

	Coefficient	Std. Error	t	P	VIF
a	17.3228	0.3063	56.5619	<0.0001	10.2020<
b	5.8411	0.2942	19.8523	<0.0001	10.2020<

Analysis of Variance:

	DF	SS	MS
Regression	2	2235.4027	1117.7014
Residual	18	1.2328	0.0685
Total	20	2236.6355	111.8318

Statistical Tests:

PRESS 1.5618

Durbin-Watson Statistic 0.8624 Failed

Normality Test Passed (P = 0.9999)

K-S Statistic = 0.0725 Significance Level = 0.9999

Constant Variance Test Passed (P = 0.9064)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	2.5322	-0.0672	-0.2568	-0.2659	-0.2589
2	4.4185	0.0916	0.3501	0.3757	0.3665
3	5.8781	0.3689	1.4095	1.5312	1.5956
4	7.0410	0.5387	2.0585	2.2282<	2.5446<
5	7.9894	-0.0127	-0.0486	-0.0521	-0.0506
6	8.7776	-0.0449	-0.1714	-0.1815	-0.1765
7	9.4431	-0.3242	-1.2387	-1.2976	-1.3245
8	10.0124	0.1051	0.4015	0.4173	0.4075
9	10.5050	-0.2205	-0.8425	-0.8709	-0.8648
10	10.9354	-0.4715	-1.8016	-1.8573	-2.0075<
11	11.3146	-0.2177	-0.8317	-0.8570	-0.8503
12	11.6514	-0.2404	-0.9185	-0.9478	-0.9450
13	11.9524	-0.1869	-0.7142	-0.7393	-0.7296
14	12.2231	-0.1350	-0.5160	-0.5366	-0.5257
15	12.4678	0.0347	0.1324	0.1385	0.1346
16	12.6901	-0.0003	-0.0011	-0.0012	-0.0011
17	12.8929	0.1773	0.6774	0.7186	0.7086
18	13.0787	0.3439	1.3142	1.4057	1.4479
19	13.2496	0.1731	0.6613	0.7136	0.7036
20	13.4072	0.2553	0.9757	1.0627	1.0668

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.0025	0.0672	-0.0695
2	0.0107	0.1313	0.1425
3	0.2111	0.1526	0.6771
4	0.4262	0.1465	1.0544
5	0.0002	0.1284	-0.0194

6	0.0020	0.1075	-0.0613
7	0.0819	0.0887	-0.4132
8	0.0070	0.0740	0.1152
9	0.0260	0.0642	-0.2265
10	0.1082	0.0590	-0.5029
11	0.0227	0.0581	-0.2113
12	0.0291	0.0609	-0.2407
13	0.0196	0.0668	-0.1952
14	0.0117	0.0752	-0.1499
15	0.0009	0.0858	0.0412
16	7.2921E-008	0.0980	-0.0004
17	0.0324	0.1115	0.2510
18	0.1424	0.1260	0.5497
19	0.0419	0.1412	0.2853
20	0.1052	0.1571	0.4605

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	2.5322	2.3896	2.6747	1.9642	3.1002
2	4.4185	4.2193	4.6177	3.8337	5.0033
3	5.8781	5.6633	6.0928	5.2878	6.4683
4	7.0410	6.8305	7.2515	6.4523	7.6297
5	7.9894	7.7924	8.1864	7.4054	8.5735
6	8.7776	8.5974	8.9579	8.1990	9.3563
7	9.4431	9.2794	9.6068	8.8694	10.0168
8	10.0124	9.8628	10.1620	9.4426	10.5822
9	10.5050	10.3657	10.6443	9.9378	11.0722
10	10.9354	10.8018	11.0690	10.3695	11.5012
11	11.3146	11.1821	11.4472	10.7491	11.8802
12	11.6514	11.5157	11.7871	11.0851	12.2177
13	11.9524	11.8103	12.0945	11.3845	12.5203
14	12.2231	12.0723	12.3739	11.6530	12.7932
15	12.4678	12.3068	12.6288	11.8949	13.0407
16	12.6901	12.5180	12.8621	12.1140	13.2662
17	12.8929	12.7093	13.0765	12.3132	13.4725
18	13.0787	12.8836	13.2738	12.4953	13.6621
19	13.2496	13.0429	13.4562	12.6622	13.8369
20	13.4072	13.1893	13.6251	12.8158	13.9986

Fit Equation Description:

[Variables]
x = col(23)
y = col(24)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 17.3228}}
b = x50(x,y,0.1) "Auto {{previous: 5.8411}}
[Equation]
f=a*x/(b+x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Options]
tolerance=1e-10
stepsize=1
iterations=200
[Constraints]

Number of Iterations Performed = 10

AQU-Columbia Deep Base Regression

Nonlinear Regression

Data Source: Data 14 in MethodsPaper.JNB
Equation: Single Rectangular, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9993	0.9986	0.9985	0.8333

	Coefficient	Std. Error	t	P	VIF
a	27.9714	0.5405	51.7489	<0.0001	4.8480<
b	2.5589	0.2171	11.7882	<0.0001	4.8480<

Analysis of Variance:

	DF	SS	MS
Regression	2	9014.9914	4507.4957
Residual	18	12.4990	0.6944
Total	20	9027.4904	451.3745

Statistical Tests:

PRESS 17.2302

Durbin-Watson Statistic 0.5041 Failed

Normality Test Passed (P = 0.8112)

K-S Statistic = 0.1381 Significance Level = 0.8112

Constant Variance Test Passed (P = 0.1378)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	7.8595	2.4393	2.9273<	3.2275<	4.8322<
2	12.2710	1.0057	1.2068	1.3639	1.3998
3	15.0953	-0.3457	-0.4149	-0.4587	-0.4484
4	17.0585	-0.6674	-0.8009	-0.8627	-0.8562
5	18.5022	-1.1935	-1.4323	-1.5125	-1.5732
6	19.6085	-0.7961	-0.9553	-0.9956	-0.9954
7	20.4834	-1.2014	-1.4417	-1.4908	-1.5475
8	21.1925	-0.0011	-0.0014	-0.0014	-0.0014
9	21.7790	-0.1214	-0.1456	-0.1498	-0.1456
10	22.2721	-0.0706	-0.0847	-0.0872	-0.0847
11	22.6924	-0.1261	-0.1513	-0.1559	-0.1517
12	23.0550	-0.2568	-0.3081	-0.3183	-0.3102
13	23.3710	-0.1185	-0.1423	-0.1474	-0.1434
14	23.6488	-0.0167	-0.0201	-0.0209	-0.0203
15	23.8950	0.1744	0.2092	0.2185	0.2126
16	24.1146	0.2143	0.2572	0.2696	0.2625
17	24.3118	0.3944	0.4733	0.4982	0.4876
18	24.4898	0.6326	0.7591	0.8023	0.7940
19	24.6513	0.4711	0.5653	0.5999	0.5890
20	24.7985	0.7078	0.8494	0.9051	0.9003

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	1.1228	0.1773	2.2436<
2	0.2579	0.2171	0.7371
3	0.0234	0.1817	-0.2113
4	0.0595	0.1380	-0.3425
5	0.1316	0.1032	-0.5337

6	0.0427	0.0794	-0.2923
7	0.0770	0.0648	-0.4072
8	5.9705E-008	0.0570	-0.0003
9	0.0006	0.0543	-0.0349
10	0.0002	0.0550	-0.0204
11	0.0008	0.0582	-0.0377
12	0.0034	0.0630	-0.0805
13	0.0008	0.0690	-0.0390
14	1.7872E-005	0.0756	-0.0058
15	0.0022	0.0827	0.0638
16	0.0036	0.0900	0.0826
17	0.0134	0.0974	0.1602
18	0.0377	0.1048	0.2717
19	0.0227	0.1121	0.2093
20	0.0555	0.1193	0.3313

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	7.8595	7.1222	8.5967	5.9599	9.7591
2	12.2710	11.4553	13.0867	10.3396	14.2024
3	15.0953	14.3490	15.8416	13.1922	16.9985
4	17.0585	16.4082	17.7087	15.1909	18.9260
5	18.5022	17.9398	19.0645	16.6634	20.3410
6	19.6085	19.1152	20.1018	17.7896	21.4274
7	20.4834	20.0378	20.9290	18.6769	22.2899
8	21.1925	20.7744	21.6107	19.3926	22.9925
9	21.7790	21.3711	22.1869	19.9814	23.5766
10	22.2721	21.8614	22.6827	20.4738	24.0703
11	22.6924	22.2701	23.1148	20.8915	24.4933
12	23.0550	22.6155	23.4945	21.2500	24.8600
13	23.3710	22.9112	23.8307	21.5609	25.1810
14	23.6488	23.1674	24.1302	21.8331	25.4645
15	23.8950	23.3915	24.3984	22.0733	25.7166
16	24.1146	23.5894	24.6399	22.2868	25.9424
17	24.3118	23.7654	24.8582	22.4778	26.1458
18	24.4898	23.9230	25.0566	22.6497	26.3300
19	24.6513	24.0651	25.2375	22.8051	26.4975
20	24.7985	24.1938	25.4031	22.9463	26.6506

Fit Equation Description:

[Variables]
x = col(23)
y = col(25)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 27.9714}}
b = x50(x,y,0.1) "Auto {{previous: 2.55895}}
[Equation]
f=a*x/(b+x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Options]
tolerance=1e-10
stepsize=1
iterations=200
[Constraints]

Number of Iterations Performed = 9

Figure 3-7. TOC_{aq} utilization efficiency (%, defined as $\Delta TOC_{aq} \times 100\% / TOC_s$) over 45 days for extraction 1 (E1), E3, and E8 of the continuous extractions using SWS-Stroubles sediment.

Figure 3-7: SWS-Stroubles Bioassay Experiment

Extractant	Day 1 Utilization (%, ΔTOC_{aq}/Extracted TOC_{aq})	Std Dev	Day 3 Utilization (%, ΔTOC_{aq}/Extracted TOC_{aq})	Std Dev	Day 8 Utilization (%, ΔTOC_{aq}/Extracted TOC_{aq})	Std Dev
0.1% Pyro	0.21	0.01	0.98	0.07	0.29	0.05
Base	3.32	0.15	0.76	0.02	0.55	0.06
0.1% Pyro Alt	0.31	0.03	3.42	0.07	0.41	0.08

Appendix B. Chapter 4

Figure 4-1. Summary of reductive dechlorination enrichment culture experiments where each subgraph illustrates the aqueous contaminant concentration over time for the following conditions: (A) MLS10 Shallow Pyrophosphate extract with the MLS12 Shallow enrichment culture, (B) MLS10 Shallow Alkali extract with the MLS 12 Shallow enrichment culture, (C) Positive control yeast extract with the MLS 12 Shallow enrichment culture, (D) MLS10 Deep Pyrophosphate extract with the MLS12 Deep enrichment culture, (E) MLS10 Deep Alkali extract with the MLS12 Deep enrichment culture, and (F) Positive control yeast extract with the MLS 12 Deep enrichment culture.

11-Aug	12-Aug	14-Aug	16-Aug	20-Aug	26-Aug	3-Sep	25-Sep
time0	time1	time2	time3	time4	time5	time6	time 7

Aqueous Concentration of Contaminant (uM)

Vial 1 — MLS10 Shallow Pyrophosphate Extract

	time0	time1	time2	time3	time4	time5	time6	time 7
PCE	0.05	0.00	0.00	0.00	0.78	0.01	0.01	0.00
TCE	1.32	1.35	0.00	0.00	1.10	1.61	2.49	3.42
cDCE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
tDCE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Vial 3 — MLS10 Shallow Alkali Extract

	time0	time1	time2	time3	time4	time5	time6	time 7
PCE	0.00	0.05	0.50	0.04	0.00	0.00	0.00	0.00
TCE	1.37	1.37	1.35	1.37	0.69	0.74	0.68	0.00
cDCE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
tDCE	0.07	0.60	0.57	0.21	0.01	0.30	0.12	2.07
VC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Vial 5 Yeast Control (MLS12 Shallow Enrichment Culture)

	time0	time1	time2	time3	time4	time5	time6	time 7
PCE	0.51	0.03	0.51	0.98	0.64	0.60	0.61	0.00
TCE	1.34	1.36	1.32	8.82	15.56	14.69	7.19	7.01
cDCE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
tDCE	0.00	0.00	0.13	0.00	0.00	0.00	0.04	0.33
VC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Vial 6 MLS10 Deep Pyrophosphate Extract

	time0	time1	time2	time3	time4	time5	time6	time 7
PCE	0.00	0.00	0.00	0.00	0.67	0.51		0.00
TCE	1.78	1.75	1.29	1.82	1.67	7.92		4.55
cDCE	0.00	0.00	0.00	0.00	0.00	0.00		0.00
tDCE	0.04	0.13	0.12	0.00	0.04	0.06		0.44
VC	0.00	0.00	0.00	0.00	0.00	0.00		0.00

Vial 8 MLS10 Deep Alkali Extract

	time0	time1	time2	time3	time4	time5	time6	time 7
PCE	0.00	0.00	0.00	0.00	0.00	0.00		0.00
TCE	1.76	1.52	1.61	1.67	0.86	0.99		0.00
cDCE	0.00	0.00	0.00	0.00	0.00	0.00		0.00
tDCE	1.25	1.36	0.00	1.68	0.47	1.08		1.58
VC	0.00	0.00	0.00	0.00	0.00	0.00		0.00

Vial 10 Yeast Control (MLS12 Deep Enrichment Culture)

	time0	time1	time2	time3	time4	time5	time6	time 7
PCE	0.00	0.00	0.00	0.06	0.00	0.51		0.00
TCE	1.69	1.77	1.72	4.73	7.43	6.84		3.05
cDCE	0.00	0.00	0.00	0.00	0.00	0.00		0.00
tDCE	0.05	0.00	0.07	0.00	0.06	0.07		0.37
VC	0.00	0.00	0.00	0.00	0.00	0.00		0.00

Table 4-1. Summary of the PCE first-order degradation rates for the enrichment cultures amendment with PCE (4 uM).

Regression for MLS12 Shallow Enrichment Culture amended with MLS10 Shallow Pyrophosphate PBOC Extracts

Nonlinear Regression

Data Source: Data 1 in Paper3.JNB
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9982	0.9964	0.9945	0.1420

	Coefficient	Std. Error	t	P	VIF
a	3.8750	0.1369	28.3124	<0.0001	1.3624
b	0.2001	0.0143	13.9647	0.0002	1.3624

Analysis of Variance:

	DF	SS	MS
Regression	2	22.0114	11.0057
Residual	4	0.0806	0.0202
Total	6	22.0920	3.6820

Statistical Tests:

PRESS 0.3037

Durbin-Watson Statistic 2.5585 Failed

Normality Test Passed (P = 0.9501)

K-S Statistic = 0.1987 Significance Level = 0.9501

Constant Variance Test Passed (P = 0.0600)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	3.8750	0.0285	0.2011	0.7566	0.7078
3	2.1257	-0.1390	-0.9789	-1.2077	-1.3121
4	1.4245	0.0966	0.6805	0.8641	0.8297
5	0.6397	0.1304	0.9188	1.0737	1.1021
6	0.1925	-0.1825	-1.2856	-1.3352	-1.5531
7	0.0388	-0.0288	-0.2030	-0.2038	-0.1774

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	3.7656	0.9294	2.5674<
3	0.3806	0.3430	-0.9479
4	0.2286	0.3798	0.6492
5	0.2107	0.2677	0.6664
6	0.0701	0.0729	-0.4354
7	0.0002	0.0073	-0.0152

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	3.8750	3.4950	4.2550	3.3275	4.4225
3	2.1257	1.8949	2.3566	1.6690	2.5825
4	1.4245	1.1816	1.6674	0.9615	1.8875
5	0.6397	0.4358	0.8437	0.1959	1.0835
6	0.1925	0.0861	0.2989	-0.2158	0.6008
7	0.0388	0.0051	0.0726	-0.3568	0.4344

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(2)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 3.87497}}
b = if(x50(x,y)-min(x)=0, 1,  -ln(.5)/(x50(x,y)-min(x)))  "Auto {{previous: 0.200139}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 7
```

Regression for MLS12 Shallow Enrichment Culture amended with MLS10 Shallow Alkali PBOC Extracts

Nonlinear Regression

Data Source: Data 1 in Paper3.JNB
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9793	0.9591	0.9428	0.4671

	Coefficient	Std. Error	t	P	VIF
a	2.8469	0.3260	8.7338	0.0003	1.5378
b	0.0867	0.0262	3.3062	0.0213	1.5378

Analysis of Variance:

	DF	SS	MS
Regression	2	25.5960	12.7980
Residual	5	1.0910	0.2182
Total	7	26.6871	3.8124

Statistical Tests:

PRESS 2.1574

Durbin-Watson Statistic 1.4128 Failed

Normality Test Passed (P = 0.7209)

K-S Statistic = 0.2473 Significance Level = 0.7209

Constant Variance Test Passed (P = 0.1209)

Power of performed test with alpha = 0.0500: 0.9954

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	2.8469	0.2512	0.5377	0.7507	0.7128
2	2.6105	0.1934	0.4140	0.5021	0.4608
3	2.1949	-0.1217	-0.2606	-0.2897	-0.2613
4	1.8454	-0.5938	-1.2711	-1.4175	-1.6393
5	1.3046	-0.3571	-0.7644	-0.9055	-0.8858
6	0.7755	0.6504	1.3923	1.6831	2.2866<
7	0.3876	0.2696	0.5771	0.6466	0.6041

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.2674	0.4869	0.6944
2	0.0593	0.3200	0.3161
3	0.0099	0.1909	-0.1269
4	0.2446	0.1958	-0.8089
5	0.1654	0.2875	-0.5626
6	0.6536	0.3157	1.5533
7	0.0533	0.2032	0.3051

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	2.8469	2.0090	3.6848	1.3827	4.3111
2	2.6105	1.9312	3.2897	1.2309	3.9900
3	2.1949	1.6703	2.7195	0.8845	3.5053
4	1.8454	1.3141	2.3768	0.5324	3.1585
5	1.3046	0.6608	1.9484	-0.0579	2.6671
6	0.7755	0.1007	1.4502	-0.6019	2.1528
7	0.3876	-0.1537	0.9289	-0.9296	1.7047

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(8)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 2.84689}}
b = if(x50(x,y)-min(x)=0, 1,  -ln(.5)/(x50(x,y)-min(x)))  "Auto {{previous: 0.0867009}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 11
```

Regression for MLS12 Shallow Enrichment Culture amended with Yeast Extract

Nonlinear Regression

Data Source: Data 1 in Paper3.JNB
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9774	0.9554	0.9375	0.8838

	Coefficient	Std. Error	t	P	VIF
a	6.4811	0.7494	8.6480	0.0003	1.4306
b	0.2326	0.0628	3.7057	0.0139	1.4306

Analysis of Variance:

	DF	SS	MS
Regression	2	83.5630	41.7815
Residual	5	3.9052	0.7810
Total	7	87.4682	12.4955

Statistical Tests:

PRESS 10.9077

Durbin-Watson Statistic 2.2215 Passed

Normality Test Passed (P = 0.9428)

K-S Statistic = 0.1882 Significance Level = 0.9428

Constant Variance Test Passed (P = 0.5453)

Power of performed test with alpha = 0.0500: 0.9940

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	6.4811	-0.4629	-0.5238	-0.9882	-0.9854
2	5.1359	0.1958	0.2215	0.2678	0.2412
3	3.2251	1.4210	1.6079	1.9676	3.7042<
4	2.0253	-1.0248	-1.1595	-1.4708	-1.7466
5	0.7986	-0.6737	-0.7623	-0.8593	-0.8325
6	0.1978	0.0729	0.0825	0.0841	0.0753
7	0.0308	0.3524	0.3987	0.3992	0.3628

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	1.2501	0.7191	-1.5766
2	0.0165	0.3157	0.1638
3	0.9631	0.3322	2.6128<
4	0.6588	0.3785	-1.3631
5	0.1000	0.2131	-0.4332
6	0.0001	0.0391	0.0152
7	0.0002	0.0023	0.0175

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	6.4811	4.5546	8.4076	3.5025	9.4598
2	5.1359	3.8595	6.4123	2.5301	7.7417
3	3.2251	1.9157	4.5346	0.6030	5.8473
4	2.0253	0.6276	3.4229	-0.6421	4.6926
5	0.7986	-0.2500	1.8473	-1.7035	3.3008
6	0.1978	-0.2514	0.6469	-2.1180	2.5135
7	0.0308	-0.0786	0.1401	-2.2437	2.3052

Fit Equation Description:

```
[Variables]
x = col(1)
y = col(3)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 6.4811}}
b = if(x50(x,y)-min(x)=0, 1,  -ln(.5)/(x50(x,y)-min(x)))  "Auto {{previous: 0.232639}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 8
```

Regression for MLS12 Deep Enrichment Culture amended with MLS10 Shallow Pyrophosphate PBOC Extracts

Nonlinear Regression

Data Source: Data 1 in Paper3.JNB
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9948	0.9896	0.9854	0.2884

	Coefficient	Std. Error	t	P	VIF
a	4.2215	0.2350	17.9662	<0.0001	1.4746
b	0.1843	0.0245	7.5202	0.0007	1.4746

Analysis of Variance:

	DF	SS	MS
Regression	2	39.5885	19.7942
Residual	5	0.4159	0.0832
Total	7	40.0043	5.7149

Statistical Tests:

PRESS 0.9749

Durbin-Watson Statistic 2.5496 Failed

Normality Test Passed (P = 0.9937)

K-S Statistic = 0.1512 Significance Level = 0.9937

Constant Variance Test Passed (P = 0.4383)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	4.2215	0.1432	0.4964	0.8561	0.8289
2	3.5111	-0.1246	-0.4321	-0.5235	-0.4816
3	2.4288	-0.3560	-1.2346	-1.4486	-1.7009
4	1.6801	0.4224	1.4646	1.8193	2.7990<
5	0.8040	0.0709	0.2458	0.2925	0.2639
6	0.2661	-0.2585	-0.8964	-0.9438	-0.9312
7	0.0011	0.0530	0.1836	0.1836	0.1648

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.7237	0.6638	1.1648
2	0.0641	0.3188	-0.3295
3	0.3954	0.2737	-1.0442
4	0.8988	0.3520	2.0627<
5	0.0178	0.2938	0.1702
6	0.0483	0.0979	-0.3068
7	2.6059E-007	1.5459E-005	0.0006

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	4.2215	3.6175	4.8255	3.2653	5.1778
2	3.5111	3.0925	3.9297	2.6598	4.3625
3	2.4288	2.0410	2.8167	1.5922	3.2655
4	1.6801	1.2403	2.1200	0.8182	2.5421
5	0.8040	0.4022	1.2058	-0.0392	1.6472
6	0.2661	0.0342	0.4981	-0.5107	1.0429
7	0.0011	-0.0019	0.0040	-0.7403	0.7424

Fit Equation Description:

```
[Variables]
x = col(27)
y = col(28)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 4.22152}}
b = if(x50(x,y)-min(x)=0, 1,  -ln(.5)/(x50(x,y)-min(x)))  "Auto {{previous: 0.184263}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 7
```

Regression for MLS12 Deep Enrichment Culture amended with MLS10 Shallow Alkali PBOC Extracts

Nonlinear Regression

Data Source: Data 1 in Paper3.JNB
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9719	0.9447	0.9078	0.6125

	Coefficient	Std. Error	t	P	VIF
a	3.1205	0.4985	6.2599	0.0082	1.3070
b	0.1504	0.0758	1.9846	0.1414	1.3070

Analysis of Variance:

	DF	SS	MS
Regression	2	19.2160	9.6080
Residual	3	1.1256	0.3752
Total	5	20.3416	4.0683

Statistical Tests:

PRESS 3.4616

Durbin-Watson Statistic 1.2111 Failed

Normality Test Passed (P = 0.3312)

K-S Statistic = 0.3937 Significance Level = 0.3312

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.8524

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	3.1205	0.3971	0.6483	1.1156	1.1908
2	2.6847	-0.3893	-0.6356	-0.8041	-0.7413
3	1.4710	-0.2641	-0.4312	-0.7157	-0.6417
4	0.3269	0.5886	0.9609	1.1697	1.2950
5	0.0036	0.6326	1.0327	1.0329	1.0506

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	1.2206	0.6623	1.6677
2	0.1941	0.3751	-0.5744
3	0.4493	0.6370	-0.8500
4	0.3297	0.3252	0.8990
5	0.0002	0.0004	0.0205

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	3.1205	1.5341	4.7069	0.6072	5.6338
2	2.6847	1.4908	3.8787	0.3988	4.9706
3	1.4710	-0.0848	3.0267	-1.0230	3.9650
4	0.3269	-0.7848	1.4385	-1.9171	2.5709
5	0.0036	-0.0345	0.0417	-1.9461	1.9533

Fit Equation Description:
[Variables]
x = col(33)
y = col(34)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2

'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 3.1205}}
b = if(x50(x,y)-min(x)=0, 1, -ln(.5)/(x50(x,y)-min(x))) "Auto {{previous: 0.150411}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 19

Regression for MLS12 Deep Enrichment Culture amended with Yeast Extract

Nonlinear Regression

Data Source: Data 1 in Paper3.JNB
Equation: Single, 2 Parameter

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9813	0.9630	0.9482	0.4905

	Coefficient	Std. Error	t	P	VIF
a	4.1667	0.4304	9.6803	0.0002	1.3875
b	0.2879	0.0688	4.1840	0.0086	1.3875

Analysis of Variance:

	DF	SS	MS
Regression	2	31.2866	15.6433
Residual	5	1.2031	0.2406
Total	7	32.4898	4.6414

Statistical Tests:

PRESS 3.8752

Durbin-Watson Statistic 3.1886 Failed

Normality Test Passed (P = 0.9956)

K-S Statistic = 0.1472 Significance Level = 0.9956

Constant Variance Test Passed (P = 0.0956)

Power of performed test with alpha = 0.0500: 0.9966

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	4.1667	0.2477	0.5050	1.0528	1.0674
2	3.1245	-0.6536	-1.3324	-1.6160	-2.0913<
3	1.7569	0.7514	1.5317	1.9681	3.7083<
4	0.9879	-0.2215	-0.4515	-0.5693	-0.5265
5	0.3124	-0.2910	-0.5932	-0.6368	-0.5942
6	0.0555	-0.0073	-0.0149	-0.0150	-0.0134
7	9.8666E-006	0.1274	0.2597	0.2597	0.2339

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	1.8548	0.7699	1.9527
2	0.6151	0.3202	-1.4354
3	1.2605	0.3943	2.9917<
4	0.0955	0.3709	-0.4043
5	0.0309	0.1324	-0.2321
6	1.4070E-006	0.0123	-0.0015
7	1.2632E-010	3.7451E-009	1.4313E-005

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	4.1667	3.0602	5.2731	2.4891	5.8442
2	3.1245	2.4109	3.8380	1.6756	4.5733
3	1.7569	0.9651	2.5487	0.2680	3.2458
4	0.9879	0.2200	1.7558	-0.4885	2.4643
5	0.3124	-0.1464	0.7711	-1.0295	1.6542
6	0.0555	-0.0846	0.1956	-1.2132	1.3243
7	9.8666E-006	-6.7302E-005	8.7035E-005	-1.2610	1.2610

Fit Equation Description:

```
[Variables]
x = col(27)
y = col(40)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
F(q)=if(size(x)>1, if(total(abs(y))>0, ape(x,log(abs(y)),1,0,1), -306), 0)
asign(q)=if(mean(q)>=0,1,-1)
[Parameters]
a = if(F(0)[1]< 307, if(F(0)[1]>-307, asign(y)*10^F(0)[1], asign(y)*10^(-307)), asign(y)*10^307) "Auto {{previous: 4.16666}}
b = if(x50(x,y)-min(x)=0, 1,  -ln(.5)/(x50(x,y)-min(x)))  "Auto {{previous: 0.287855}}
[Equation]
f = a*exp(-b*x)
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
[Options]
tolerance = 1e-10
stepsize = 1
iterations=200

Number of Iterations Performed = 6
```

Figure 4-2. Aqueous hydrogen concentrations (nM) over time for pyrophosphate and alkali extracts on shallow (8-10 ft) and deep (20.5-22 ft) MLS10 sediment from NABLC.

Days	Pyrophosphate Shallow	Std Dev	Alkali Shallow	Std Dev	Pyrophosphate Deep	Std Dev	Alkali Deep	Std Dev
1	0.14	0.05	7.19	0.41	0.16	0.04	16.78	2.55
9	0.16	0.05	15.59	0.63	0.44	0.04	13.35	1.40
14	0.00	0.00	19.49	3.42	0.31	0.16	29.48	10.22
28	0.04	0.07	34.45	3.59	0.07	0.12	52.75	4.03
42	0.00	0.00	20.81	1.67	0.00	0.00	50.40	6.95

Table 4-3. Summary of percent carbon utilized by the aerobic and anaerobic bioassays for pyrophosphate and alkali extractions of MLS10 and MLS20 sediment samples.

MLS10 Shallow Aerobic (Wet oxidiation TOC machine)

Sample	**Initial TOC (mg/L)**	**DTOC (mg/L)**	**Std Dev**	**% utilized**	**Std Dev**
0.1% Pyro	9.183	1.122	0.26	12.22%	2.80%
Base/Pyro	6.016	1.420	0.19	23.60%	3.20%

MLS10 Deep Aerobic

Sample	**Initial TOC (mg/L)**	**DTOC (mg/L)**	**Std Dev**	**% utilized**	**Std Dev**
0.1% Pyro	64.175	20.607	0.85	32.11%	1.33%
Base/Pyro	32.581	17.500	1.21	53.71%	3.71%

MLS10 Anaerobic (Combustion TOC machine)

		Pyrophosphate Extraction			**Alkali Extraction**		
Sample	**Depth**	**Carbon Extracted (mg/L)**	**Carbon Utilized (mg/L)**	**% Carbon Utilized**	**Carbon Extracted (mg/L)**	**Carbon Utilized (mg/L)**	**% Carbon Utilized**
MLS10	8-10 ft	10.01	NA	NA	7.58	3.50	24.0%
MLS10	20.5-22 ft	17.34	5.49	31.7%	28.23	NA	NA

Appendix C. Chapter 5

Figure 5-2. Summary of ground-water redox parameters and contaminant concentrations from July 1999 at NABLC. For (A) Sulfate concentrations and (B) Methane concentrations, each graph illustrates the distribution of the redox parameter over the depth of the multi level sampler (MLS) for MLS10, MLS12, MLS22, and MLS20. For (C) MLS12 and (D) MLS20, the chloroethene-contaminant concentrations are shown over the depth of MLS. Depth is shown in terms of elevation.

Ground-water redox parameter data.

Elevation	MLS10		Elevation	MLS12		Elevation	MLS22		Elevation	MLS20	
	Sulfate	Methane		Sulfate	Methane		Sulfate	Methane		Sulfate	Metha
-9.3	21.7	122.4	-9.8	11.1	95.8	-9.9	31.4		-10.8	10.1	504.
-6.8	91.4	12.3	-7.3	41.9	80.3	-7.4	30.2		-8.3	30.1	550.
-4.3	91.8	2.4	-4.8	69.4	1.2	-2.4	41.5		-5.8	18.9	150.
-1.8	110	1.6	-2.3	109.0	0.6	0.1	49.6		-3.3	17.9	195.
0.7	79.5	0.9	-0.2	109.0	27.2	2.6	57.5		-0.8	5.48	187
3.2	80.7	0.3	2.7	109.0	61.1	5.1	39.3		1.7	5.85	1789
7.8	121	0.0									

Chloroethene data for MLS12

Elevation	MLS12			
	PCE	TCE	cDCE	VC
-9.8	12000	1300	110	BDL
-7.3	4400	280	BDL	BDL
-4.8	130	31	BDL	BDL
-2.3	81	33	BDL	BDL
-0.2	340	260	220	6.8
2.7	490	270	310	57

Chloroethene data for MLS20

Elevation	MLS20			
	PCE	TCE	cDCE	VC
-10.8	1200	350	110	BDL
-8.3	3400	630	130	BDL
-5.8	860	86	30	BDL
-3.3	13	1.2	BDL	BDL
-0.8	6.8	1.4	BDL	BDL
1.7	6.8	4.2	1.6	BDL

Figure 5-3. Extracted aqueous carbon concentration (mg/kg) as total PBOC for the pyrophosphate and alkali extractions over all depths for MLS10, MLS12, MLS20, and MLS22.

Use the data in Table 5-1.

Figure 5-4. Extracted aqueous carbon concentration (mg/kg) for the pyrophosphate and alkali extractions at the shallow depth (8-10 ft) for MLS10, MLS12, MLS20, and MLS22.

Use the data in Table 5-1.

Figure 5-5. Extracted aqueous carbon concentration (mg/kg) for the pyrophosphate and alkali extractions at deep depth (20-22 ft) for MLS10, MLS12, MLS20, and MLS22.

Use the data in Table 5-1.

Figure 5-6. Summary of the of aerobic bioassay results in terms of carbon utilized (ΔTOC_{aq} mg/L) versus extracted TOC_{aq} concentration (mg/L) for the pyrophosphate and alkali extracts across all depths at MLS10, MLS12, MLS20, and MLS22. Due to the use of the log-log scale, all bioassays that exhibited no utilization of TOC_{aq} are shown on the x-axis at an artificial value of ΔTOC=0.1 mg/L.

Bioassay results

Sample	Depth (ft)	Pyrophosphate Extraction		Alakli Extraction	
		Carbon Extracted (mg/L)	Carbon Utilized (mg/L)	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)
MLS10	8-10	9.18	1.12	6.02	1.42
	10-12	6.88	2.11	2.62	0.00
	19-20.5	29.70	3.34	7.54	0.95
	20.5-22	64.17	20.61	32.58	17.50
MLS12	8-10	3.02	0.79	0.87	0.00
	10-12	6.67	0.00	2.78	0.00
	16-18	6.56	0.00	0.54	0.00
	20	27.60	0.00	14.98	3.90
MLS20	8-10	2.26	0.00	1.61	0.00
	10-12	2.89	0.00	1.79	0.00
	16-18	6.71	0.00	2.84	0.00
	22	36.53	10.06	15.74	6.80
MLS22	8-10	12.17	1.76	7.52	2.24
	10-12	5.13	0.88	1.81	0.00
	18-20	6.95	0.73	1.42	0.00
	22	46.04	4.60	14.32	2.68

Table 5-2. Summary of aerobic bioassay results in terms of carbon extracted (mg/L), carbon utilized (mg/L) and % carbon utilized for the pyrophosphate and alkali extractions at MLS10 and MLS20.

Use the data in Figure 5-6.

Figure 5-7. Cumulative headspace CO_2 generation shown as mg C produced per bioassay sample for pyrophosphate extract bioassays of MLS10 and MLS20 at the shallow and deep depths.

MLS10 Shallow Pyrophosphate CO2-C (mgC)

Time	Avg CO2	St Dev
0	5.50E-03	0.00E+00
37	2.79E-03	6.58E-03
54	1.23E-02	4.66E-03
67	1.57E-02	7.03E-03
79	2.13E-02	8.92E-03

MLS10 Deep Pyrophosphate CO2-C (mgC)

Time	Avg CO2	St Dev
0	5.50E-03	0.00E+00
37	6.01E-02	7.05E-03
54	1.01E-01	1.29E-02
67	1.24E-01	6.71E-03
79	1.37E-01	8.00E-03

MLS20 Shallow Pyrophosphate CO2-C(mg)

Time	Avg CO2	St Dev
0	7.17E-03	0.00E+00
14	8.36E-03	1.35E-02
24	8.36E-03	1.20E-02
41	9.80E-03	1.18E-02
53	1.26E-02	9.85E-03
76	2.13E-02	1.07E-02

MLS20 Deep Pyrophosphate CO2-C(mg)

Time	Avg CO2	St Dev
0	0.00E+00	0.00E+00
14	3.79E-02	2.56E-03
24	7.35E-02	1.26E-03
41	9.60E-02	1.53E-03
53	1.05E-01	4.88E-03
76	1.21E-01	4.49E-03

Appendix D. Chapter 6

Figure 6-1. PBOC (mg/kg) versus the solid-phase total organic carbon (TOC_s, mg/kg) for the six study sites. Data is labeled and encircled for each site.
Figure 6-2. Summary of PBOC (mg/kg) versus the sediment total organic carbon (TOC_s, mg/kg) for five of the six study sites (the NAS data are omitted).
Figure 6-3. Soft carbon content (mg/kg) versus the sediment total organic carbon (TOC_s, mg/kg) for aquifer sediment containing soft carbon.
Figure 6-4. PBOC (mg/kg) versus the soft carbon content (mg/kg) for aquifer sediment for the four of six study sites (the NAS and DoD data are omitted).
Figure 6-5. TOC_{aq} (mg/kg) versus the sediment total organic carbon (TOC_s, mg/kg) for the six study sites where graph (A) included sites with active reductive dechlorination and graph (B) included sites with minimal reductive dechlorination.

Data for Figure 6-1 through Figure 6-5 come from the following tables which are divided between sites with active and minimal reductive dechlorination.

Active Dechlorination Sites
NABLC

Sample Location	Depth (ft)	Extractions (mg/kg)				TOCs (mg/kg)	Soft Carbon (mg/kg)
		Pyro	Std Dev	Alkali	Std dev		
MLS10	8-10	39.9	2.0	13.8	1.3	187	149
	10-12	34.4	1.5	7.5	0.6	71	56
	16-19	149.6	8.8	26.1	4.3	405	337
	20-22	324.2	14.5	111.6	11.3	1185	724
MLS12	8-10	15.5	0.4	3.0	0.3	130	68
	10-12	34.1	5.1	9.4	0.2	148	BDL
	16-19	33.2	4.1	1.8	0.8	105	77
	20-22	142.8	2.0	51.8	4.2	832	764
MLS20	8-10	6.1	0.2	2.2	0.3	0	BDL
	10-12	9.6	2.3	2.8	0.2	207	177
	16-19	29.6	6.0	6.5	1.2	687	648
	20-22	182.2	10.7	51.0	2.7	670	624
MLS22	8-10	62.1	4.8	26.0	0.7	462	274
	10-12	26.3	5.1	6.1	0.8	72	54
	16-19	35.9	0.9	4.8	0.7	97	24
	20-22	268.0	13.4	55.6	3.2	1176	1019
MW6	8-10	12.7	1.7	2.2	0.4	136	77
	10-12	5.6	0.1	0.9	0.4	59	BDL
	18-20	204.4	12.0	53.3	0.3	869	746
MIP08	23-24	115.0	5.6	195.1	12.6	1130	383
MIP10	23-24	125.5	2.5	219.9	28.1	981	372

NSB

Sample	Depth (ft)	Extractions (mg/kg)				TOCs (mg/kg)	Soft Carbon (mg/kg)
		Pyro	Std Dev	Alkali	Std dev		
Outcrop	3m	3166.6	127.0	11003.1	872.0	16020	9093
KBA13A	10-13	359.5	3.4	174.6	36.9	936	264
5/11/2002	3m	958.8	155.0	1404.5	133.0	3642	1785

MCRD

Sample Location	Depth	Extractions (mg/kg)				TOCs (mg/kg)	Soft Carbon (mg/kg)
		Pyro	Std Dev	Alkali	Std dev		
MW21-SU	3-8 ft	419.7	15.0	65.3	5.2	1034	871
MW21-SL	10-15 ft	159.3	8.1	310.9	18.6	1408	850
MW22-SU	3-8 ft	370.9	5.7	147.3	0.5	1571	1185
MW22-SL	10-15 ft	217.4	2.6	355.6	48.9	1786	976

Minimal Reductive Dechlorination Sites

Site Name	Aquifer Sediment Sample ID	Depth (ft)	Extractions (mg/kg)				TOCs (mg/kg)	Soft Carbon (mg/kg)
			Pyro	Std Dev	Alkali	std dev		
DOD	10CO39	37-38	16.0	2.7	2.1	0.1	23.6	BDL
		46-47	13.1	1.0	2.6	0.1	22.9	BDL
		58-59	1.7	1.0	2.5	0.2	23.0	BDL
	10CO41	43-44	23.9	1.3	2.5	0.3	34.9	BDL
		45-46	28.8	1.8	2.8	0.3	0.1	BDL
		59-60	2.0	0.4	1.3	0.1	0.1	BDL
	10CO49	41.5-42	7.5	0.4	2.9	0.1	0.1	74
		45-46	5.9	0.5	2.2	0.5	0.1	BDL
		49.5-50	3.8	0.3	1.4	0.2	0.1	BDL
		54-54.5	5.5	0.5	1.8	0.3	0.1	BDL
		59-60	5.2	0.4	1.8	0.2	23.0	BDL
ACP	PESMW10B	68-70 ft	7.7	0.7	118.9	38.0	223	165
	PESMW13A	38-40 ft	18.0	1.1	175.5	95.9	182	NA
NAS	799E115I	43-45 ft	129.3	7.5	172.3	7.2	12794	5499
	799E151U	28-30 ft	151.3	22.2	204.0	80.4	40724	12555
	799E152I	43-45 ft	218.1	25.7	223.2	73.2	26379	6992

Figure 6-6. Summary of bioassays for sites with active reductive dechlorination where the delta ΔTOC_{aq} (mg/L) was the maximum utilization of aqueous TOC over 45 days in the bioassays and the extracted TOC concentrations were the initial TOC concentrations in the bioassays. Due to the use of the log-log scale, all bioassays that exhibited no utilization of TOC_{aq} are shown on the x-axis at an artificial value of ΔTOC_{aq}=0.1 mg/L.

Figure 6-7. Summary of bioassays for sites with minimal reductive dechlorination where the ΔTOC_{aq} (mg/L) was the maximum utilization of aqueous TOC over 45 days in the bioassays and the extracted TOC concentrations were the initial TOC concentrations in the bioassays. Due to the use of the log-log scale, all bioassays that exhibited no utilization of TOC_{aq} are shown on the x-axis at an artificial value of ΔTOC_{aq}=0.1 mg/L.

Data for Figure 6-6 and Figure 6-7 are shown in the following the tables.

Active Reductive Dechlorination
NABLC Site

Sample	Depth (ft)	Pyro Extraction		Alkali Extraction	
		Carbon Extracted (mg/L)	Carbon Utilized (mg/L)	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)
MLS10	8-10	9.2	1.1	6.0	1.4
	10-12	6.9	2.1	2.6	0.1
	19-20.5	29.7	3.3	7.5	0.9
	20.5-22	64.2	20.6	32.6	17.5
MLS12	8-10	3.0	0.8	0.9	0.1
	10-12	6.7	0.1	2.8	0.1
	16-18	6.6	0.1	0.5	0.1
	20	27.6	0.1	15.0	3.9
MLS20	8-10	2.3	0.1	1.6	0.1
	10-12	2.9	0.1	1.8	0.1
	16-18	6.7	0.1	2.8	0.1
	22	36.5	10.1	15.7	6.8
MLS22	8-10	12.2	1.8	7.5	2.2
	10-12	5.1	0.9	1.8	0.1
	18-20	7.0	0.7	1.4	0.1
	22	46.0	4.6	14.3	2.7
MW6	8-10	2.5	0.1	0.6	0.1
	10-12	1.1	0.1	0.2	0.1
	18-20	40.3	0.1	15.7	5.4
MIP08	23-24	22.7	6.3	57.1	18.0
MIP10	23-24	19.2	0.1	49.8	0.1

NSB Site

Sample	Pyro Extraction		Alkali Extraction	
	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)
Outcrop	617.9	386.1	3187.4	1343.1
KBA13A	70.3	49.2	50.4	27.3
5/11/2002	185.1	129.7	400.6	256.9

MCRD

Sample	Depth	Pyro Extraction		Alkali Extraction	
		Carbon Extracted (mg/L)	Carbon Utilized (mg/L)	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)
MW21-SU	3-8 ft	64.3	0.1	14.9	0.1
MW21-SL	10-15 ft	19.8	0.1	70.1	13.3
MW22-SU	3-8 ft	87.2	0.1	33.5	0.1
MW22-SL	10-15 ft	33.3	0.1	81.0	24.7

Minimal Reductive Dechlorination

Site Name	Sample	Depth	Pyro Extraction		Alkali Extraction	
			Carbon Extracted (mg/L)	Carbon Utilized (mg/L)	Carbon Extracted (mg/L)	Carbon Utilized (mg/L)
DOD	10CO39	37-38	3.3	0.1	0.5	0.1
	10CO39	46-47	3.2	0.1	0.7	0.1
	10CO39	58-59	0.8	0.1	0.6	0.1
	10CO41	43-44	4.6	1.5	0.6	0.1
	10CO41	45-46	5.5	1.9	0.7	0.1
	10CO41	59-60	0.6	0.1	0.6	0.1
	10CO49	41.5-42	1.5	0.1	0.8	0.1
	10CO49	45-46	1.3	0.1	0.8	0.1
	10CO49	49.5-50	0.9	0.1	0.6	0.1
	10CO49	54-54.5	1.2	0.1	0.7	0.1
	10CO49	59-60	1.1	0.1	0.7	0.1
ACP	PESMW10B	68-70 ft	1.2	0.1	26.6	24.4
	PESMW13A	38-40 ft	2.7	0.1	39.1	36.8
NAS	799E115I	43-45 ft	30.7	8.9	27.1	20.2
	799E151U	28-30 ft	28.2	8.8	24.3	19.8
	799E152I	43-45 ft	30.7	5.3	26.6	16.1

Vita

Heather Veith Rectanus

Education

Ph.D. Civil Engineering, Environmental Engineering with Geoenvironmental Emphasis, Dec 2006
Virginia Tech, GPA 3.97
Future Professoriate Graduate Certificate
Dissertation: Sustainability of reductive dechlorination at chlorinated solvent contaminated sites: Methods to evaluate biodegradable natural organic carbon.
Co-Advisors: John T. Novak and Mark A. Widdowson

M.S. Civil Engineering, Environmental Engineering in Geoenvironmental Track, Dec 2000
Virginia Tech, GPA 3.95
Thesis: Assessment of Intrinsic Bioremediation at a PCE Contaminated Site
Co-Advisors: John T. Novak and Mark A. Widdowson

B.S. Nuclear Engineering, May 1998
B.A. German, May 1998
Kansas State University, Summa Cum Laude, GPA 3.97

Honors and Awards

Graduate Assistance in Areas of National Need (GAANN) Fellowship, 2002-2004
Virginia Tech Charles E. Via Fellowship, 2000-2003
National Science Foundation Fellowship, 1998-2001
Virginia Tech Master's Via Fellowship, 1998-1999
Nuclear Engineering Scholar, ORI for Science and Education, 1997
American Nuclear Society Scholar, 1995-1997
National Academy for Nuclear Training Scholar, 1996-1998

Professional Experience

Research
Doctoral Research Assistant, Virginia Tech, Jan 2001-present

- Developed and validated a method to estimate the potentially bioavailable organic carbon in sediments
- Organized and conducted groundwater and soil sampling field trips
- Trained and supervised master level students in the laboratory and field
- Conducted and contributed to model-based, theoretical research addressing the time to remediation of NAPL contamination

Master's Research Assistant, Virginia Tech, May 1998-Dec 2000

- Performed, as part of a team, groundwater field sampling including dissolved hydrogen measurements
- Conducted microcosm studies on PCE contaminated aquifer sediment using anaerobic techniques
- Modeled PCE degradation via the reductive dechlorination package of SEAM3D
- Analyzed groundwater data at chloroethene-contaminated site

Undergraduate Research Assistant, KSU, Jan 1994-Sept 1994

- Designed and conducted organic chemistry experiments to synthesize Taxol C-13 side chain
- Separated reaction end products on silica gel columns and evaluated end products with ^{1}H NMR

Nuclear

- Senior Reactor Operator, KSU TRIGA Mark II Nuclear Reactor Facility, Aug 1995-May 1998
- Designed and implemented mechanical system upgrades for license renewal requirements
- Conducted public information tours of reactor facility to various audience levels
- Updated and wrote safety analysis reports for license renewal requirements
- Operated reactor for isotope production, maintained mechanical systems, and monitored water quality parameters

Language

- Study Abroad Scholar, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland, Oct 1994-July 1995
- Studied Mechanical Engineering and Nuclear Physics at the ETH

Research Interests

Impact of natural organic carbon in environmental systems
Subsurface contaminant interactions and remediation strategies
Monitored natural attenuation
Radionuclide contaminant immobilization and radioactive waste disposal

Teaching Experience

Co-Instructor, Fluid Mechanics for Civil and Environmental Engineers, Virginia Tech

- Lectures; with Dr. Widdowson
- Spring 2005, Spring 2003, Summer 2001

Teaching Assistant, Water Resources Engineering Laboratory, Virginia Tech

- Laboratory lectures and experiments
- Fall 2001, Spring 2002

Teaching Assistant, Nuclear Reactor Operations, KSU

- Laboratory lectures and experiments
- Spring 1996-1998

University Service

Mentor for High School students working on nitrate contamination of groundwater, 2001-2002
Civil and Environmental Engineering representative to Graduate Student Assembly, 2002-2003
Environmental Engineering Program Recruitment Weekend Committee Chair, 2002-present
Student Action Committee for Environmental Engineering Program, President, 2003-2004

Licensure

OSHA 29 CFR 1910.120 – 40 Hour Initial Training
Intern Engineer State of Kansas, June 1998
Senior Reactor Operator (SOP-70187), 1 October 1996
Reactor Operator (OP-70105), 4 October 1994

Affiliations

National Ground Water Association, Student Member
American Chemical Society, Student Member
American Nuclear Society, Student Member
Tau Beta Pi, Phi Kappa Phi, Phi Beta Kappa, Alpha Nu Sigma

Presentations and Publications

H.V. Rectanus, M.A. Widdowson, J.T. Novak, and F.H. Chapelle. "A Method for Quantifying Bioavailable Organic Carbon in Aquifer Sediments." Oral presentation and conference proceedings, Third European Bioremediation Conference, July 4-7, 2005, Chania, Crete, Greece.

H.V. Rectanus, M.A. Widdowson, J.T. Novak, and F.H. Chapelle. "Method Development for Quantifying Bioavailable Organic Carbon in Aquifer Sediments." Poster presentation, Eighth International In Situ and On-Site Bioremediation, June 6-9, 2005, Baltimore, MD.

M.A. Widdowson, F. H. Chapelle, H.V. Rectanus, and J.S. Brauner. "Relationship Between NAPL Mass and Remediation Time Using Monitored Natural Attenuation." First International Congress on Petroleum Contaminated Soils, Sediments & Water, August 14-17, 2001, London, England.

M.A. Widdowson, H.V. Rectanus, M.J. Higgins, D.F. Berry, and J.T. Novak. "Integrated Assessment of MNA at a PCE Contaminated Site." Poster presentation and conference proceedings, In Situ and On-Site Bioremediation, June 4-7, 2001, San Diego, CA.

M.A. Widdowson, J.T. Novak, D.F. Berry, H.V. Rectanus, and F.Y. Wang. "Spatial Variation in Reductive Dechlorination of a PCE-Contaminated Aquifer." Remediation of Chlorinated and Recalcitrant Compounds, May 22-25, 2000, Monterey, CA.

D.F. Berry, M.J. Higgins, H.V. Rectanus, M.A. Widdowson, and J.T. Novak. "Investigation of Intrinsic Bioremediation at NAB Little Creek, Site12: Microbiological Assessment." 1999 International Symposium on Subsurface Microbiology, August 22-27, 1999, Vail, CO.