Laboratory Evaluation of Natural and Enhanced Remediation of Chlorinated Ethenes in Fractured Sandstone

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LABORATORY EVALUATION OF NATURAL AND ENHANCED REMEDIATION OF CHLORINATED ETHENES IN FRACTURED SANDSTONE

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Environmental Engineering and Earth Science

by
Rong Yu
August 2017

Accepted by:
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Dr. Ronald W. Falta
Dr. Lawrence C. Murdoch
Dr. Kevin T. Finneran
ABSTRACT

Chlorinated ethenes are common industrial chemicals and among the most frequently detected groundwater contaminants. Behavior and remediation of chlorinated ethenes in unconsolidated aquifers composed of granular materials (e.g., sand, silt and gravel) has been extensively studied for several decades. Nevertheless, it was not until the end of twentieth century that the role of matrix diffusion in plume persistence gained widespread acceptance. Matrix diffusion commonly occurs in complicated hydrogeological settings such as fractured sedimentary bedrock aquifers, where the permeable fractures act as the major conduit for groundwater flow and the less permeable but high capacity matrix acts as the primary storage place for contaminants.

A fractured sandstone aquifer at an industrial site in southern California is contaminated with trichloroethene (TCE) to depths in excess of 244 m. Field monitoring data and previous microcosm studies suggest that TCE is undergoing reductive dechlorination to mainly cis-1,2-dichloroethene (cDCE) and additional attenuation through slow abiotic transformation that generates acetylene, CO₂ and soluble compounds (referred to as non-strippable residue, or NSR). Biostimulation has been identified as a promising technology to treat this site by enhancing both biological and abiotic degradation. The objectives of this study were to determine the effect of biostimulation on reductive dechlorination of TCE to cDCE and other transformation pathways using crushed rock microcosms; to develop and operate a novel type of microcosm composed of intact rock cores to evaluate natural attenuation and biostimulation in a fracture-matrix system; and to develop and validate a numerical
model for reactive transport of chlorinated ethenes in intact rock core microcosms, and then use the model to determine rate constants for natural attenuation.

To achieve the first objective, over 500 crushed rock microcosms were constructed, using TCE and cDCE, and eleven treatments covering various types of amendments. In addition to the conventional headspace and liquid phase analyses, $^{14}$C-labeled TCE and cDCE were used to quantify the rate and extent of product formation; enrichment in $\delta^{13}$C was measured in microcosms without $^{14}$C added.

Lactate, hydrogen release compound® (HRC), and emulsified vegetable oil (EVO) significantly enhanced the rate of TCE reduction to cDCE. Lactate also stimulated reductive dechlorination of cDCE to vinyl chloride (VC) and ethene, suggesting the presence of indigenous *Dehalococcoides* that are not active in situ due to donor-limited conditions. Illumina sequencing and qPCR analysis demonstrated that *Geobacter* spp. are responsible for reductive dechlorination of TCE to cDCE and *Dehalococcoides* spp. for reduction of cDCE to VC and ethene. The rate of TCE reduction to cDCE and cDCE to VC was faster than for VC to ethene, suggesting that *Dehalococcoides* perform the final dechlorination step co-metabolically. This was subsequently confirmed in enrichment cultures fed with VC where no activity was observed, while TCE and cDCE were readily reduced to ethene. Abiotic transformation of TCE and cDCE was observed based on accumulation of $^{14}$C daughter products and $\delta^{13}$C enrichment in the absence of reductive dechlorination. Electron donor and sulfate amendments did not enhance abiotic transformation, in spite of repeated sulfate consumption. Accumulation of $^{14}$CO$_2$ plus $^{14}$C-NSR in unamended microcosms was used to determine pseudo-first order abiotic
transformation rates of $0.038 \text{ yr}^{-1}$ for TCE and $0.044 \text{ yr}^{-1}$ for cDCE, corresponding to half-lives of 18 and 16 yr, respectively.

Since crushing disturbs the surface area of the rock, it was unclear the extent to which crushed rock microcosms deviate from the behavior in undisturbed rock. The second objective was to learn about the processes in undisturbed rock using intact core microcosms. A novel microcosm design was developed. Each microcosm consisted of a sandstone core inserted between stainless steel end caps, and sealed inside layers of Teflon tape, a Teflon sleeve and an outer stainless steel case. Site groundwater amended with TCE, bromide and resazurin was forced through the rock under pressure to contaminate the core. One end cap was hollowed out to create a groundwater reservoir and was connected with two valves for sampling. Paired cores with similar characteristics were set up, one serving as an unamended control and the other as a treatment biostimulated with lactate. Lactate was chosen because it was the most effective electron donor for enhancing reductive dechlorination in crushed rock microcosms. Weekly sampling was conducted that also served the purpose of lactate delivery and to generate a groundwater flow over the simulated fracture. Samples were analyzed for TCE and volatile daughter products, anions, organic acids, and pH. Evaluation of $\delta^{13}\text{C}$ was carried out every 3-4 months. Lactate addition created low redox conditions and stimulated sulfate reduction as well as reductive dechlorination. However, only TCE to cDCE degradation occurred, indicating a low population or absence of indigenous Dehalococcoides, potentially caused by their heterogeneous distribution at the site. Biostimulation significantly enhanced the contaminant removal rate by increasing the
concentration gradient of TCE between the matrix and fracture and converting TCE to its more mobile daughter product, cDCE.

Enrichment in δ¹³C was observed for TCE in rock core microcosms that did not undergo a discernible level of reductive dechlorination, and for cDCE formed via reductive dechlorination of TCE. This outcome indicated that an alternative transformation pathway for TCE and cDCE occurred, as observed in the crushed rock microcosms.

To achieve the third objective, a numerical model was developed in a 2D radial symmetrical system using COMSOL Multiphysics. The model simulates diffusion, biotic/abiotic reactions, sampling and isotopic fractionation within dual porosity media (i.e., rock-fracture). The model was successfully calibrated with three sets of experimental data (TCE/cDCE, bromide and δ¹³C) from the intact core microcosms, and generated site-specific parameters including rock diffusivity, Monod kinetic constants, and abiotic transformation rates. This greatly elevated the relevancy and applicability of intact core microcosms to evaluation of transformation processes that occur in the field. Sensitivity analyses indicated that parameters such as matrix diffusivity, maximum specific growth rates, and decay coefficients play key roles in controlling the TCE and cDCE concentration. Also, abiotic enrichment factors have a significant impact on predicting the rates of TCE and cDCE transformation. Model simulations indicated that abiotic transformation is governed by reactions in the core, while reductive dechlorination occurred in both the chamber and the core. Abiotic transformation rates predicted by the model based on intact rock core microcosms correspond to half-lives of
37 to 88 yr for TCE and 37 to 63 yr for cDCE. These rates were longer than those determined with $^{14}$C-labeled compounds in the crushed rock microcosms, likely due to an increase in surface area during crushing.
DEDICATION

This work is dedicated to

My parents, Jiang Liqun and Yu Shaogong

&

My grandma, Han Shuheng

&

All my relatives and friends

for their continuous love and support
ACKNOWLEDGEMENTS

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A special thanks to lab managers Anne Cumming and Dave Lipscomb for their help with instruments trouble shooting. Also I want to thank Rodney Merck, Rodney Morgan and Dock Houston for their invaluable contribution in building the intact core microcosms.

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Finally, I greatly appreciate the financial and logistical support for this research by Richard Andrachek and MWH Global (now part of Stantec).
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$^{14}$C</td>
<td>carbon-14 labeled (radioisotope) compound</td>
</tr>
<tr>
<td>$\delta^{13}$C</td>
<td>carbon-13 to carbon-12 isotope ratio, expressed in per mil</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>isotope enrichment factor</td>
</tr>
<tr>
<td>AC</td>
<td>autoclave control</td>
</tr>
<tr>
<td>AS</td>
<td>autoclaved plus sulfide</td>
</tr>
<tr>
<td>cDCE</td>
<td>cis-1,2-dichloroethene</td>
</tr>
<tr>
<td>COD</td>
<td>chemical oxygen demand</td>
</tr>
<tr>
<td>CSIA</td>
<td>compound-specific isotope analysis</td>
</tr>
<tr>
<td>D. Dehalococcoides</td>
<td></td>
</tr>
<tr>
<td>E or EVO</td>
<td>emulsified vegetable oil</td>
</tr>
<tr>
<td>ES</td>
<td>emulsified vegetable oil plus sulfate</td>
</tr>
<tr>
<td>H or HRC</td>
<td>hydrogen release compound</td>
</tr>
<tr>
<td>HS</td>
<td>hydrogen release compound plus sulfate</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>KIE</td>
<td>kinetic isotopic effect</td>
</tr>
<tr>
<td>L</td>
<td>lactate</td>
</tr>
<tr>
<td>LS</td>
<td>lactate plus sulfate</td>
</tr>
<tr>
<td>MSM</td>
<td>mineral salts medium</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>NSR</td>
<td>non-strippable residue</td>
</tr>
<tr>
<td>P.</td>
<td><em>Pelobacter</em></td>
</tr>
<tr>
<td>PCE</td>
<td>tetrachloroethene</td>
</tr>
<tr>
<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>S</td>
<td>sulfate</td>
</tr>
<tr>
<td>SS</td>
<td>stainless steel</td>
</tr>
<tr>
<td>TCE</td>
<td>trichloroethene</td>
</tr>
<tr>
<td>tDCE</td>
<td><em>trans</em>-1,2-dichloroethene</td>
</tr>
<tr>
<td>U or UN</td>
<td>unamended</td>
</tr>
<tr>
<td>UNF</td>
<td>unidentified fraction</td>
</tr>
<tr>
<td>v/v</td>
<td>volume/volume</td>
</tr>
<tr>
<td>VC</td>
<td>vinyl chloride</td>
</tr>
<tr>
<td>VOCs</td>
<td>volatile organic compounds</td>
</tr>
<tr>
<td>WC</td>
<td>water control</td>
</tr>
</tbody>
</table>
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1. INTRODUCTION

1.1. Overview

Chlorinated ethenes are common industrial chemicals and among the most frequently detected groundwater contaminants. The behavior and remediation of chlorinated ethenes in unconsolidated aquifers composed of granular materials (e.g., sand, silt and gravel) has been extensively studied for several decades. However, it was not until the beginning of this century that awareness developed around the significant contribution of matrix diffusion to plume persistence, even after removal of source zones containing dense nonaqueous phase liquids (DNAPL). As a result, research is increasingly focused on the mechanisms of contaminant transport and destruction in complex hydrogeological settings under the influence of matrix diffusion. One example includes widely distributed fractured bedrock aquifers in the United States.

Monitored natural attenuation (MNA) and biostimulation are two cost-effective approaches to treat contaminated fractured bedrock sites. Bench-scale microcosm studies are often conducted in addition to field studies in search of evidence in support of natural attenuation or to evaluate active remediation options. To perform controlled experiments in a laboratory, the norm has been to use crushed rock samples and groundwater in microcosms. This allows for simulation of the interaction between the rock minerals and microbes with contaminants, and for characterization of individual biological, chemical and physical processes. However, critical processes that are prevalent in complicated hydrogeological settings may be overlooked. The development of a more representative
experimental system to study fractured bedrock, and the application of model simulations for data interpretation and prediction of long-term trends, offers the promise of advancing the state of knowledge regarding remediation of complicated sites. The focus of this dissertation is on use of biostimulation to enhance biotic and abiotic degradation of chlorinated ethenes in fractured bedrock, including development of a novel intact rock core microcosm to more realistically represent in situ conditions.

The following sections provide a review of remediation alternatives for fractured bedrock aquifers, degradation pathways for chlorinated ethenes that may occur in fractured bedrock, and methods to evaluate transformation reactions and rates within fractured bedrock. A description of the specific site that is the subject of this dissertation is provided, followed by the objectives for this research.

1.2. **Remediation of Fractured Bedrock Aquifer**

Fractured bedrock aquifers are often susceptible to industrial pollution and recalcitrant to remediation. Groundwater flow is controlled by two distinct components of the aquifer: the fracture network acts as the major flow conduit and the less permeable but high capacity rock matrix as the primary storage place. Exchange of contaminant between the active groundwater in fractures and the nearly immobile groundwater in the rock matrix is dominated by matrix diffusion; consequently, plume behavior does not follow the models commonly used to simulate porous medium for homogenous aquifers.

Site and plume characterization during in situ remediation is difficult; consequently, many CERCLA and RCRA sites with fractured bedrock choose a hydraulic containment strategy, e.g., pump and treat, to avoid the need to accurately locate the
contaminant (1). Although employed frequently, pump and treat may be less effective in fractured bedrock than in unconsolidated aquifers. Contaminant is mainly removed from interconnected fractures but less from the rock matrix, which is the primary contaminant source/sink. Continuous back diffusion from the matrix into the active fracture flow may sustain the plume and significantly extend the remediation time frame (2-4). Over the past decade, increasing attention has gone to development of in situ treatment techniques, including bioremediation, chemical oxidation and reduction, and thermal treatment (1).

Bioremediation is an appealing technique for treating fractured bedrock because of its low cost and sustainability. Biostimulation to enhance indigenous microbial activity is the most common practice, usually involving injection of substrate into the subsurface to adjust the redox level, satisfy electron donor demand, and supply necessary carbon sources and nutrients. Bioaugmentation becomes necessary when functional microbes are lacking in situ. Bioremediation relies on direct contact between the amendment and contaminant, which can be hard to achieve in fractured bedrock. Amendment distribution is highly affected by the tortuous flow system. Whether amendments can penetrate the low permeability rock to react with absorbed contaminant is largely controlled by pore throat sizes. Using a slow-release electron donor that dissolves slowly into the groundwater and gradually ferments into more effective substrates may prevent it from being washed out too fast. Studies by Ross et al. (5-7) also demonstrated the possibility of cultivating a biofilm on the fracture surface as a barrier against contaminant migration.

In situ chemical oxidation is accomplished by injecting or inducing strong chemical oxidizers directly into the fractured aquifer to destroy contaminants in place.
Common agents include sodium and potassium permanganate, Fenton's reagent/hydrogen peroxide, sodium persulfate, ozone, perozone/perozone (ozone activated with hydrogen peroxide) and percarbonate (1, 8). Compared to other technologies, chemical oxidation usually produce no significant waste and achieves contaminant reduction within relatively short time frames (e.g., weeks or months) (9). Again, complicated hydrogeology and low permeability zones in fractured bedrock aquifers can impede the distribution and mixing of chemicals. Besides, the effectiveness is contingent on the relatively short life span of the oxidant, which ranges from minutes (e.g., hydrogen peroxide) to months (e.g., permanganate) (1). For these reasons, chemical oxidation is predominantly used to address contaminants in the source area and saturated zone where mass is more concentrated (1, 8-9).

In situ chemical reduction is the mirror process of chemical oxidation, which encompasses a range of techniques including addition of a liquid reducing agent into the subsurface, or placing a permeable reactive barrier of reductants in the path of a contaminant plume (also a containment method) (10-11). Zero valent iron (ZVI) is the most widely used reducing agent; one advantage of ZVI is its greater longevity (years), making it a promising candidate to deal with back diffusion (1). Delivery of chemical reductant faces the same challenges of heterogeneity and low permeability, and ensuring a continuous barrier across all fractures is difficult (1).

In situ thermal remediation involves heating subsurface groundwater and the vadose zone to facilitate volatilization or destruction of contaminant, usually followed by vapor extraction and ex situ treatment (1). Heat transfer does not depend on active
groundwater flow and thus is less sensitive to geological heterogeneity (12). There are three major heating technologies: steam enhanced extraction, electrical resistance heating, and thermal conduction heating (13). Among these options, in situ thermal desorption is the most efficient one in heating low permeability media and it is least affected by active fracture flow (13); therefore, it is a preferred technique for fractured bedrock. Thermal treatment usually achieves contaminant removal in a short period, but can be cost-intensive. A potential concern for low permeability zones is that heating can increase the pressure and raise the boiling point of the contaminants, so that longer boiling times or higher temperatures may be required (12, 14).

Due to the intrinsic complexity of fractured bedrock, even the most aggressive methods may not achieve sufficient mass removal within a reasonable timeframe. Since complete mass removal is so challenging, the remediation strategy for such sites is often focused on plume control and long-term mass destruction, for which monitored natural attenuation (MNA) is a cost-effective alternative. MNA relies on naturally occurring processes, such as biotic and abiotic degradation, dilution, and evaporation to reduce contaminant concentrations in the groundwater. Matrix diffusion and dispersion can also contribute to natural attenuation by retarding the plume (15). MNA often occurs at much slower rates than engineered processes and may not be suitable as a sole remedy for heavily contaminated sites (15). However, for trapped mass in low permeability zones or residual levels of contaminant, MNA may be a sustainable long term remedy. Moreover, natural attenuation is likely to occur in low permeability zones, because 1) reducing conditions are common in the matrix due a lack of groundwater inflow; 2) sedimentary
rock often acts as a reservoir for surplus organic matter and reduced minerals that facilitate reductive transformation reactions; and 3) long retention times in the matrix can ultimately lead to significant mass reduction (16).

To facilitate decision making among available remediation techniques, a fundamental understanding of individual attenuation processes and their potential interactions in the context of fractured bedrock is needed. The next section looks into major degradation pathways for chlorinated ethenes under anaerobic conditions, their likelihood in fractured bedrock aquifers, and their potential role in natural attenuation or engineered remediation. In the section that follows, available methodologies to document and quantify these pathways are reviewed. Emphasis is placed on methods to determine reliable rate constants (or half lives) for the degradation processes, which is a key parameter in models used to predict plume stability (17-18).

1.3. Degradation Pathways for Chlorinated Ethenes

Degradation of chlorinated ethenes under anaerobic conditions encompasses reductive dechlorination (i.e., hydrogenolysis), β-elimination, α-elimination, and hydrogenation (19-20), as shown in Figure 1-1. A review of each pathway follows.

1.3.1 Reductive Dechlorination

Reductive dechlorination is the dominant biological degradation pathway for tetrachloroethene (PCE), trichloroethene (TCE), cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC) under anaerobic conditions. Anaerobic oxidation has been reported for cDCE and VC (21-23). There is currently no evidence that anaerobic oxidation occurs with PCE and TCE. Proving that anaerobic oxidation occurs in the field is difficult
because the ultimate daughter products are chloride ions and CO₂, which are not easily measured in the field unless the initial concentrations of cDCE and VC are sufficiently high, which is rarely the case.

Dechlorinating bacteria, either indigenous or introduced through bioaugmentation, are the key component of bioremediation. Several microorganisms capable of reductively dechlorinating TCE and other chlorinated ethenes have been identified and/or isolated. Some of them are only able to reduce TCE to its immediate daughter product, cDCE, while others can reduce it to ethene and occasionally ethane. The *Dehalococcoides* genus is known to dechlorinate PCE and TCE to ethene. *Dehalococcoides mccartyi* strain 195 (24) and *Dehalococcoides* sp. strain FL2 (25) can reduce TCE to VC metabolically, then cometabolically convert VC to ethene. *Dehalococcoides* strain GT (26), strain VS (27), and *Dehalococcoides mccartyi* strain BTF08 (28) are capable of chlororespiring TCE all the way to ethene. *Dehalococcoides* strain BAV-1 (29) is capable of chlororespiring cDCE to VC and ethene. The only known genus other than *Dehalococcoides* that is capable of dechlorinating DCE isomers and VC is *Dehalogenimonas*. With ethanol + lactate as electron donors, *Dehalogenimonas* sp. are capable of chlororespiring TCE all the way to ethene. *Dehalococcoides* strain BAV-1 (29) is capable of chlororespiring cDCE to VC and ethene. The only known genus other than *Dehalococcoides* that is capable of dechlorinating DCE isomers and VC is *Dehalogenimonas*. With ethanol plus lactate as electron donors, *Dehalogenimonas* sp. strain WBC-2 can dechlorinate *trans*-1,2-dichloroethene (tDCE) to ethene (30-31).
Figure 1-1. Pathways for biotic and biotic transformation of chlorinated ethenes.
Other microorganisms that chlororespire TCE to cDCE but are incapable of further dechlorination have been isolated or enriched, including *Desulfitobacterium* sp. (32-36), *Dehalobacter restrictus* (37-39), *Sulfurospirillum* sp. (40-41), *Desulfuromonas* sp. (42-44), *Desulfomonile tiedjei* (45) and *Geobacter lovleyi* (26, 46). Growth of these chlororespiring microbes occurs under strictly anaerobic conditions. By contrast, a facultative aerobe (*Enterobacter agglomerans* strain MS-1) has been isolated that is also capable of reductively dechlorinating TCE to cDCE (47). Strain MS-1 grows anaerobically with several electron donors, including acetate, lactate and pyruvate (47).

Microbiological characterization of fractured bedrock aquifers has focused primarily on the biomass suspended in groundwater samples and largely overlooked the microbes attached to the fracture surface and in matrix pores. However, the majority of the contaminant ends up in the rock matrix, so it is important to characterize microbial communities inside the pore spaces and evaluate their contribution to overall plume attenuation. Average pore-throat sizes are larger than 2 μm in conventional reservoir rocks, around 0.03-2.0 μm for tight-gas sandstones, and 0.005-0.1 μm in shales (48). Since the diameter of most microbial cells is on the order of 1 μm, their ability to migrate into and grow within the matrix is limited (49). Previous studies suggest that the metabolic activity of subsurface bacteria may not be sustained in rock with pore throats smaller than 0.2 μm, though viable bacteria can still be maintained and stimulated (50). On the other hand, several studies have detected microorganisms capable of TCE degradation growing within matrices with sufficiently large pores and pore throat sizes. Takeuchi et al. (51) detected biological dechlorination activity in an organic-rich clayey
aquitard contaminated with PCE. Using qPCR analysis and closely-spaced sampling of cores up to 8 m long, a large population of Dehalococcoides, anaerobic hydrogen producing bacteria, and the key reductase gene vcrA, were detected in the clayey layer. Lima et al. (52) demonstrated the presence of dechlorinating microorganisms in rock matrices at a site where a fractured sandstone-dolostone is contaminated with chlorinated and aromatic hydrocarbons. The microbial community was characterized based on depth-discrete, high-frequency sampling along a 98 m continuous rock core. Chlororespiring microbes (including Dehalococcoides and Dehalobacter) were detected in the rock away from fracture surfaces. These studies demonstrated that biodegradation within the rock matrix should be considered when evaluating the potential of natural attenuation or remediation at fractured rock sites.

Microbes need an electron donor, an electron acceptor and a carbon source to grow (with exceptions, e.g., fermentative microbes do not need an electron acceptor). During chlororespiration, TCE serves as the electron acceptor and hydrogen is considered the universal electron donor used by Dehalococcoides and other microbes (53-54). Compounds that can serve as carbon sources include acetate (25), pyruvate (47), and ethanol (33), some of which may also serve as electron donors (26, 46). Often, naturally occurring organic matter in situ can serve as an electron donor and carbon source. When in deficit, amendments can be supplied (biostimulation).

1.3.2 Abiotic Transformations

When decreases in TCE are observed without a corresponding accumulation of reductive dechlorination products, or when reduction of TCE does not significantly
progress beyond cDCE or VC, it is important to determine if a pathway exists for the removal of cDCE or VC other than via biotic reductive dechlorination.

Interest in the contribution of abiotic degradation pathways to natural attenuation continues to grow. Several lines of evidence are commonly used in support of abiotic degradation, including: 1) documentation of plume stability; 2) mineralogical analysis to identify iron bearing minerals that potentially mediate abiotic transformation; 3) monitoring for diagnostic reaction products (e.g., acetylene); 4) compound specific isotope analysis (CSIA) to assess the degree of enrichment; and 5) use of microcosm studies to specifically examine abiotic reactions and their rates (55). Identification of abiotic pathways can be complicated by the coexistence of biotic processes, which can either overshadow the existence of abiotic degradation or share the same daughter products, e.g., ethene (Fig. 1-1).

The presence of iron-bearing minerals may be an indicator of abiotic transformation. Often minerals such as magnetite and iron sulfides are present in the rock matrix at concentrations too low to be detected by traditional methods such as X-ray diffraction. Nevertheless, concentrations of minerals as low as 1% in the rock matrix can make a significant contribution to abiotic processes. These minerals are vulnerable to oxidation in the presence of air, so taking solid samples may also compromise their measurement. Geochemical modeling and geophysical analyses may help predict the composition and reactivity of minerals present in the rock (56-58).

Acetylene is an important reaction product for both abiotic transformation of TCE and PCE. Approximately 70-80% of TCE that is abiotically degraded by iron sulfide is
recovered as acetylene (56). Acetylene is also produced by a wide variety of other reactive mineral species, such as green rusts. In theory, acetylene should serve as a broad, unambiguous marker for abiotic degradation of chlorinated ethenes in groundwater in the same way that cDCE is an indicator of biotic reductive dechlorination. However, acetylene has not played a similar role for abiotic processes, in large part because it appears to degrade quickly in groundwater. Abiotic degradation of chlorinated ethenes by iron sulfides has been confirmed at several sites even when acetylene has not been detected.

One of the reasons that acetylene does not typically accumulate in groundwater may be that it undergoes biodegradation. Schink (59) demonstrated that acetylene may be fermented by *Pelobacter acetylenicus* to acetic acid and ethanol (or the respective higher acids and alcohols). The first step, hydration of acetylene to acetaldehyde by acetylene hydratase, appears to be a highly exergonic reaction:

\[
\text{C}_2\text{H}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CHO} \quad \Delta G^\circ' = -111.9 \text{ kJ/mol acetylene} \ (1)
\]

Further disproportionation to acetate and ethanol is less favorable, but still exergonic:

\[
2\text{CH}_3\text{CHO} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{OH} + \text{CH}_3\text{COO}^- + \text{H}^+ \quad \Delta G^\circ'' = -17.3 \text{ kJ/mol aldehyde} \ (2)
\]

A recent study by Mao et al. (60) showed that *Pelobacter* strain SFB93 can ferment acetylene to hydrogen and acetate. This process may support reductive dechlorination by removing acetylene, an inhibitory factor, and providing the required electron donor (H₂) and a carbon source (acetate).
Another factor that limits detection of acetylene in groundwater relates to field sampling techniques. Because of its high Henry’s Law coefficient, acetylene readily volatilizes. Conventional sampling methods withdraw groundwater through pumping, which is associated with significant volatilization problems. Passive groundwater sampling devices such as the Snap Sampler™ may improve the detection of acetylene (61).

Products other than acetylene from abiotic and/or coupled abiotic/biotic degradation of chlorinated ethenes have been reported. Darlington et al. (62-63) proposed that TCE and cDCE are transformed to CO₂ and non-chlorinated organic acids (primarily glycolate, formate, and acetate) by iron-containing minerals in crushed sandstone (Fig. 1-2). Because CO₂ and organic acids may also be present in groundwater from naturally occurring sources, their detection required the use of ¹⁴C-labeled TCE and cDCE. Use of ¹⁴C-labelled compounds is definitive but limited to laboratory studies.

CSIA is a powerful tool to evaluate degradation of contaminants. During biotic and abiotic degradation, bonds with ¹²C are broken preferentially over bonds with ¹³C, resulting in an enrichment of ¹³C in the parent compound. This process is referred to as carbon isotope fractionation and is quantified using the carbon isotope signature, δ¹³C. The relationship between isotope fractionation and reaction extent is expressed in terms of an enrichment factor (ε), the magnitude of which varies widely depending on the transformation process. Tables 1-1 to 1-3 summarize reported δ¹³C enrichment factors for biotic and abiotic transformation of cDCE and TCE. If sufficient degradation occurs to establish enrichment factors, they can be used as evidence to identify biotic or abiotic
transformation. This is relatively straightforward in unconsolidated aquifers where there is a well-defined flow path. CSIA applied to downgradient samples can be compared to upgradient samples and a rate of enrichment can be quantified. Obtaining enrichment factors for transformation within fractured rock systems is more challenging, since it is almost impossible to trace the movement of water from one point to another and obtain evidence of enrichment along a flow path.

Figure 1-2. Pathways proposed by Darlington et al. (63) based on transformation of $^{14}$C-TCE: A = abiotic; B = biotic, A/B = abiotic and/or biotic, [H] = $H^+ + e^-$. 
Table 1-1. $\delta^{13}$C enrichment factors ($\varepsilon$) for cDCE via different degradation pathways.

<table>
<thead>
<tr>
<th>$\varepsilon$ (%)</th>
<th>$R^2$</th>
<th>Organism/Chemical/Processes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-21.7 \pm 1.8$</td>
<td>N/A</td>
<td>Abiotic with iron</td>
<td>(64)</td>
</tr>
<tr>
<td>$-14.4$</td>
<td>0.99</td>
<td>Abiotic with iron</td>
<td>(65)</td>
</tr>
<tr>
<td>$-6.9 \pm 2.0$ to $16.0 \pm 1.1$</td>
<td>0.95</td>
<td>Peerless and Connelly irons</td>
<td>(66)</td>
</tr>
<tr>
<td>$-21.1 \pm 0.6$</td>
<td>0.99</td>
<td>$\text{KMnO}_4$</td>
<td>(67)</td>
</tr>
<tr>
<td>$-16.9 \pm 1.4$</td>
<td>0.98</td>
<td>$\text{Dehalococcoides}$ sp. strain BAV1</td>
<td>(68)</td>
</tr>
<tr>
<td>$-14.9 \pm 0.5$</td>
<td>0.99</td>
<td>$\text{Dehalococcoides}$ sp. strain BAV1</td>
<td>(69)</td>
</tr>
<tr>
<td>$-15.8 \pm 1.1$</td>
<td>0.97</td>
<td>$\text{Dehalococcoides}$ sp. strain FL2</td>
<td>(69)</td>
</tr>
<tr>
<td>$21.6 \pm 1.3$</td>
<td>0.97</td>
<td>$\text{Dehalococcoides}$ sp. strain GT</td>
<td>(69)</td>
</tr>
<tr>
<td>$17.6 \pm 2.7$</td>
<td>0.89</td>
<td>$\text{Dehalococcoides}$ sp. strain VS</td>
<td>(69)</td>
</tr>
<tr>
<td>$-16.1; -14.1$</td>
<td>0.991; 0.924</td>
<td>$\text{Dehalococcoides}$–containing KB−1 consortium</td>
<td>(70)</td>
</tr>
<tr>
<td>$-20.4 \pm 1.2$</td>
<td>0.94</td>
<td>$\text{Dehalococcoides}$–containing KB−1 consortium</td>
<td>(71)</td>
</tr>
<tr>
<td>$-29.7 \pm 1.6$</td>
<td>0.99</td>
<td>$\text{Dehalococcoides}$–containing ANAS consortium</td>
<td>(68)</td>
</tr>
<tr>
<td>$-25.3 \pm 1.0$</td>
<td>0.99</td>
<td>BDI consortium</td>
<td>(69)</td>
</tr>
<tr>
<td>$-12$</td>
<td>N/A</td>
<td>Anaerobic microcosms constructed from a PCE contaminated site</td>
<td>(72)</td>
</tr>
<tr>
<td>$-19.9 \pm 1.5$</td>
<td>0.984</td>
<td>Anaerobic enrichment culture</td>
<td>(73)</td>
</tr>
<tr>
<td>$-9.8 \pm 1.7; \quad -8.8 \pm 1.0; \quad -7.1 \pm 0.9; \quad -8.2 \pm 3.5$</td>
<td>0.977; 0.967; 0.971; 0.687</td>
<td>Aerobic cometabolic degradation by an enrichment culture (12–14°C)</td>
<td>(74)</td>
</tr>
<tr>
<td>$-1.10 \pm 0.77$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by Pseudomonas putida F1</td>
<td>(75)</td>
</tr>
<tr>
<td>$-1.32 \pm 0.45$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by $P. \text{fluorescens}$ CFS215</td>
<td>(75)</td>
</tr>
<tr>
<td>$-0.89 \pm 0.51$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by $R. \text{pickettii}$ PKO1</td>
<td>(75)</td>
</tr>
<tr>
<td>$-17.4$ to $-22.4$</td>
<td>0.97</td>
<td>Aerobic oxidation by $Polaromonas$ sp. strain JS666</td>
<td>(76)</td>
</tr>
</tbody>
</table>
Table 1-2. $\delta^{13}$C enrichment factors ($\varepsilon$) for TCE via different degradation pathways.

<table>
<thead>
<tr>
<th>$\varepsilon$ (%)</th>
<th>$R^2$</th>
<th>Organism/Chemical/Processes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-33.4 \pm 1.5$</td>
<td>N/A</td>
<td>FeS–mediated reductive dechlorination (pH = 8)</td>
<td>(77)</td>
</tr>
<tr>
<td>$-27.9 \pm 1.3$</td>
<td>N/A</td>
<td>FeS–mediated reductive dechlorination (pH = 9)</td>
<td>(77)</td>
</tr>
<tr>
<td>$-9.6$</td>
<td>N/A</td>
<td>FeS–mediated reductive dechlorination (pH = 7.3)</td>
<td>(78)</td>
</tr>
<tr>
<td>$-21.7 \pm 1.0$</td>
<td>N/A</td>
<td>Pyrite (pH=8)</td>
<td>(79)</td>
</tr>
<tr>
<td>$-39 \pm 12$</td>
<td>N/A</td>
<td>Magnetite–mediated reductive dechlorination</td>
<td>(77)</td>
</tr>
<tr>
<td>$-23.0 \pm 1.8$</td>
<td>N/A</td>
<td>Chloride green rust–mediated reductive dechlorination</td>
<td>(77)</td>
</tr>
<tr>
<td>$-10.1$</td>
<td>N/A</td>
<td>ZVI filings</td>
<td>(80)</td>
</tr>
<tr>
<td>$-23.5 \pm 2.8$</td>
<td>N/A</td>
<td>nanoscale ZVI (Fe$^{0}$)</td>
<td>(64)</td>
</tr>
<tr>
<td>$-20.9 \pm 1.1$ to $-26.5 \pm 1.5$</td>
<td>N/A</td>
<td>nanoscale ZVI (Fe$^{2+}$)</td>
<td>(64)</td>
</tr>
<tr>
<td>$-8.6$</td>
<td>0.97</td>
<td>metallic iron</td>
<td>(65)</td>
</tr>
<tr>
<td>$-16.7$</td>
<td>N/A</td>
<td>cast and autoclaved electrolytic iron</td>
<td>(81)</td>
</tr>
<tr>
<td>$-7.5 \pm 0.4$ to $-13.9 \pm 1.3$</td>
<td>0.94</td>
<td>Peerless and Connelly irons</td>
<td>(66)</td>
</tr>
<tr>
<td>$-26.3 \pm 2.4$ to $-18.5 \pm 0.8$</td>
<td>$&gt; 0.97$</td>
<td>K$<em>{2}$MnO$</em>{4}$</td>
<td>(67)</td>
</tr>
<tr>
<td>$-15.4 \pm 2.1$</td>
<td>0.96</td>
<td>Vitamin B$_{12}$</td>
<td>(82)</td>
</tr>
<tr>
<td>$-17.2$ to $-16.6$</td>
<td>0.99</td>
<td>Vitamin B$_{12}$</td>
<td>(83)</td>
</tr>
<tr>
<td>16.1 $\pm 0.9$</td>
<td>N/A</td>
<td>Vitamin B$_{12}$</td>
<td>(84)</td>
</tr>
<tr>
<td>15.0</td>
<td>0.96</td>
<td>Vitamin B$_{12}$</td>
<td>(85)</td>
</tr>
<tr>
<td>21.3$\pm 0.5$</td>
<td>N/A</td>
<td>Cobaloxime</td>
<td>(84)</td>
</tr>
<tr>
<td>18.5$\pm 2.8$</td>
<td>0.96</td>
<td>Norpseudo-B$_{12}$</td>
<td>(85)</td>
</tr>
<tr>
<td>15.1$\pm 2.7$</td>
<td>0.96</td>
<td>Nor-B$_{12}$</td>
<td>(85)</td>
</tr>
<tr>
<td>15.0$\pm 2.0$</td>
<td>0.98</td>
<td>Cyano-B$_{12}$</td>
<td>(85)</td>
</tr>
<tr>
<td>16.5$\pm 0.7$</td>
<td>1.0</td>
<td>Dicyanocobinamide</td>
<td>(85)</td>
</tr>
<tr>
<td>$-8.5 \pm 0.6$</td>
<td>0.98</td>
<td>$G$. lovleyi strain SZ</td>
<td>(86)</td>
</tr>
<tr>
<td>12.2$\pm 0.5$</td>
<td>N/A</td>
<td>$G$. lovleyi strain SZ</td>
<td>(84)</td>
</tr>
<tr>
<td>$-9.3 \pm 0.6$</td>
<td>0.97</td>
<td>$G$. lovleyi strain SZ</td>
<td>(87)</td>
</tr>
</tbody>
</table>
Table 1-3, continued. $\delta^{13}$C enrichment factors ($\varepsilon$) for TCE via different degradation pathway.

<table>
<thead>
<tr>
<th>$\varepsilon$ (‰)</th>
<th>$R^2$</th>
<th>Organism/Chemical/Processes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-8.0 \pm 0.4$</td>
<td>0.98</td>
<td><em>Dehalococcoides</em> sp. strain FL2</td>
<td>(69)</td>
</tr>
<tr>
<td>$-13.7 \pm 1.8$</td>
<td>0.95</td>
<td><em>Dehalococcoides mccartyi</em> 195</td>
<td>(86)</td>
</tr>
<tr>
<td>$-9.6 \pm 0.4$</td>
<td>0.99</td>
<td><em>Dehalococcoides mccartyi</em> 195</td>
<td>(68)</td>
</tr>
<tr>
<td>$-3.3 \pm 0.3$</td>
<td>0.98</td>
<td><em>Dehalococcoides restrictus</em> strain PER-K23</td>
<td>(68)</td>
</tr>
<tr>
<td>$-8.3 \pm 0.9$</td>
<td>0.99</td>
<td><em>Dehalococcoides restrictus</em> strain PER-K23</td>
<td>(87)</td>
</tr>
<tr>
<td>$-11.2 \pm 2.6$</td>
<td>0.96</td>
<td><em>Dehalococcoides</em> strain CBDB1</td>
<td>(88)</td>
</tr>
<tr>
<td>$-6.6; -2.5$</td>
<td>1.0; 0.91</td>
<td><em>Dehalococcoides</em>-containing KB-1 consortium</td>
<td>(70)</td>
</tr>
<tr>
<td>$-13.8 \pm 0.7$</td>
<td>0.98</td>
<td><em>Dehalococcoides</em>-containing KB-1 consortium</td>
<td>(71)</td>
</tr>
<tr>
<td>$-16.0 \pm 0.6$</td>
<td>0.99</td>
<td><em>Dehalococcoides</em>-containing ANAS consortium</td>
<td>(68)</td>
</tr>
<tr>
<td>$-4.1 \pm 0.5$</td>
<td>N/A</td>
<td><em>Desulfuromonas michiganensis</em> strain BB1</td>
<td>(77)</td>
</tr>
<tr>
<td>$-3.5 \pm 0.2$</td>
<td>0.99</td>
<td><em>Desulfuromonas michiganensis</em></td>
<td>(86)</td>
</tr>
<tr>
<td>$-4.4 \pm 0.3$ to $-7.1 \pm 0.4$</td>
<td>0.98</td>
<td><em>Desulfuromonas michiganensis</em></td>
<td>(87)</td>
</tr>
<tr>
<td>$-12.8 \pm 1.6$</td>
<td>N/A</td>
<td><em>Sulfurospirillum multivorans</em></td>
<td>(77)</td>
</tr>
<tr>
<td>$-12.6 \pm 0.5$</td>
<td>N/A</td>
<td><em>Sulfurospirillum multivorans</em></td>
<td>(78)</td>
</tr>
<tr>
<td>$-16.4 \pm 1.5$</td>
<td>0.97</td>
<td><em>Sulfurospirillum multivorans</em></td>
<td>(68)</td>
</tr>
<tr>
<td>$-18.7 \pm 4.2$</td>
<td>0.84</td>
<td><em>Sulfurospirillum multivorans</em></td>
<td>(82)</td>
</tr>
<tr>
<td>$-18.9 \pm 1.0$</td>
<td>0.99</td>
<td><em>Sulfurospirillum halorespirans</em></td>
<td>(82)</td>
</tr>
<tr>
<td>$-12.2 \pm 2.3$</td>
<td>0.88</td>
<td><em>Desulfotobacterium hafniense</em> strain PCE-S</td>
<td>(82)</td>
</tr>
<tr>
<td>$-9.1 \pm 0.6$</td>
<td>N/A</td>
<td><em>Desulfotobacterium hafniense</em> Y51</td>
<td>(84)</td>
</tr>
<tr>
<td>$-15.3 \pm 0.8$</td>
<td>N/A</td>
<td>bacterial consortium (BioDechlor Inoculum)</td>
<td>(77)</td>
</tr>
<tr>
<td>$-7.1$</td>
<td>0.98</td>
<td>Pinellas consortium</td>
<td>(89)</td>
</tr>
<tr>
<td>$-7.1 \pm 0.4$ to $-10.0 \pm 0.8$</td>
<td>N/A</td>
<td>Ethanol-enrichment culture prepared from anaerobic digester sludge</td>
<td>(90)</td>
</tr>
<tr>
<td>$-4$</td>
<td>N/A</td>
<td>Anaerobic microcosms constructed from a PCE contaminated site</td>
<td>(72)</td>
</tr>
<tr>
<td>$-18.2 \pm 0.7$</td>
<td>0.95</td>
<td>Aerobic biodegradation by <em>Burkholderia cepacia</em> G4</td>
<td>(91)</td>
</tr>
<tr>
<td>$-1.1 \pm 0.3$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by <em>Methylosinus trichosporium</em> OB3b</td>
<td>(92)</td>
</tr>
<tr>
<td>$-13.82 \pm 1.55$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by <em>Pseudomonas putida</em> F1</td>
<td>(75)</td>
</tr>
<tr>
<td>$-14.70 \pm 3.02$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by <em>P. fluorescens</em> CFS215</td>
<td>(75)</td>
</tr>
<tr>
<td>$-14.40 \pm 6.44$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by <em>P. mendocina</em> KR1</td>
<td>(75)</td>
</tr>
<tr>
<td>$-11.60 \pm 4.11$</td>
<td>N/A</td>
<td>Aerobic cometabolic biodegradation by <em>Ralstonia pickettii</em> PKO1</td>
<td>(75)</td>
</tr>
</tbody>
</table>
1.3.3 Biogeochemical Transformation

Degradation of chlorinated ethenes through combined biological and abiotic pathways is commonly referred to as biogeochemical transformation. It often achieves better overall performance than each process individually, since one process supports the other or they are mutually beneficial. For example, abiotic degradation of chlorinated ethene can be stimulated via a process called BiRD (biogeochemical reductive dechlorination) or BMAD (biologically mediated abiotic degradation). It proceeds via three steps: 1) Organic compounds are provided to promote the growth of native sulfate-reducing bacteria (SRB); 2) HS\(^-\) from SRB respiration results in the precipitation of iron sulfides (FeS) or other reducing minerals; and 3) FeS chemically reduces chlorinated ethenes (93-94). Another example is the EHC\(^\circledast\) in situ chemical reduction technology, whereby a mixture of controlled-release carbon source, ZVI and nutrients is provided to create a strongly reduced environment, which then stimulates rapid and complete dechlorination through biotic and abiotic pathways (20, 95-96).

1.4. Methodologies

As mentioned in section 1.2, a variety of treatment strategies exist for fractured bedrock aquifers, such as pump-and-treat, biostimulation/bioaugmentation, chemical oxidation or reduction, thermal remediation, and MNA. Natural attenuation, which is likely to occur to some extent, needs to be evaluated prior to any treatment decision. For sites where rapid biological degradation is absent or incomplete, attention may be focused on relatively slower processes such as mineral-mediated abiotic transformation, which has the potential of retarding and reducing a plume in the context of matrix
diffusion. Rates of abiotic transformation for TCE and cDCE mediated by some metals or minerals are summarized in Table 1-4; the daughter products are also listed. It should be noted that the values listed were estimated based on minerals, not rock samples containing minerals; therefore, the rates are much higher compared to those occurring in situ.

Obtaining in situ rates in fractured bedrock is essential for remediation planning and may be achieved with a combination of field study, laboratory evaluation, and model simulation, as reviewed in the rest of this section. The research presented in this dissertation also included the development of a new laboratory method to measure the extent and rate of natural and enhanced remediation of TCE in fractured bedrock.

1.4.1 Field Monitoring

Unlike unconsolidated aquifers in which the temporal change in concentration can be calculated based on the concentration gradient over a certain distance and the groundwater speed, documenting trends in fractured bedrock aquifers is considerably complicated by flow heterogeneity. Furthermore, matrix diffusion can remove mass from the fracture flow system, making historical concentration decreases in monitoring well a less compelling line of evidence for in situ degradation. CSIA can be a valuable tool for identifying contaminant source and documenting in situ processes. However, it tends to provide a yes-or-no answer to the question of whether or not degradation is occurring, rather than accurate information on rates of degradation.
Table 1-4. Abiotic transformation products and rate constants mediated by iron-bearing minerals (97).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Metal or metal compound + amendments</th>
<th>Products</th>
<th>pseudo-first-order rate ( (s^{-1}) )</th>
<th>half life (yr)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCE</td>
<td>( Fe^0 )</td>
<td>dichloroethenes</td>
<td>2.36E-06</td>
<td>0.009</td>
<td>(65)</td>
</tr>
<tr>
<td>TCE</td>
<td>( Fe^0 )</td>
<td>acetylene, dichloroethenes</td>
<td>1.30E-06</td>
<td>0.017</td>
<td>(98)</td>
</tr>
<tr>
<td>TCE</td>
<td>nano-Pd/Fe</td>
<td>ethene, ethane</td>
<td>2.20E-06</td>
<td>0.010</td>
<td>(99)</td>
</tr>
<tr>
<td>TCE</td>
<td>( FeS_2 )</td>
<td>ethene, cDCE</td>
<td>1.03E-05</td>
<td>0.002</td>
<td>(100)</td>
</tr>
<tr>
<td>TCE</td>
<td>( FeS_2 )</td>
<td>acetylene</td>
<td>4.44E-06</td>
<td>0.005</td>
<td>(101)</td>
</tr>
<tr>
<td>TCE</td>
<td>FeS</td>
<td>acetylene, cDCE</td>
<td>3.33E-07</td>
<td>0.066</td>
<td>(102)</td>
</tr>
<tr>
<td>TCE</td>
<td>chloride green rust</td>
<td>acetylene, ethene, cDCE</td>
<td>1.69E-06</td>
<td>0.013</td>
<td>(100)</td>
</tr>
<tr>
<td>TCE</td>
<td>sulfate green rust</td>
<td>acetylene, ethene</td>
<td>6.04E-07</td>
<td>0.036</td>
<td>(103)</td>
</tr>
<tr>
<td>cDCE</td>
<td>( Fe^0 )</td>
<td>VC</td>
<td>1.51E-06</td>
<td>0.015</td>
<td>(65)</td>
</tr>
<tr>
<td>cDCE</td>
<td>nano-Pd/Fe</td>
<td>ethene, ethane</td>
<td>2.20E-06</td>
<td>0.010</td>
<td>(99)</td>
</tr>
<tr>
<td>cDCE</td>
<td>sulfate green rust</td>
<td>acetylene, ethene</td>
<td>3.69E-07</td>
<td>0.060</td>
<td>(103)</td>
</tr>
</tbody>
</table>
Geochemical modeling using historical groundwater geochemical parameters can be used as a predictive measure to determine the composition of minerals present in the soil and rock matrix. For example, geochemical modeling was conducted to determine if iron sulfide minerals were precipitating in a biowall at Altus Air Force Base (56). Modeling showed that groundwater in the biowall was saturated with iron sulfides, and the model determined $E_{h}$ values in one well were comparable to measured values.

Geophysical methods have potential to non-invasively screen for the presence of magnetite and iron sulfides in situ, as these minerals have strong signatures in terms of magnetic susceptibility and complex electrical resistivity (57-58). Iron minerals show a broad range of magnetic susceptibility responses between a highly positive response for ferrimagnetic minerals such as magnetite to a low response for antiferromagnetic minerals such as pyrite (104-105). Thus, magnetic susceptibility might be used as a tool to diagnose iron mineral transformations associated with the abiotic degradation of chloroethenes. Complex electrical resistivity is a second geophysical measurement with high sensitivity to iron minerals, particularly magnetite (58). This technique has also recently been investigated as an approach for the characterization of biotically mediated iron mineral cycling at hydrocarbon contaminated sites (58, 106). Other recent research has demonstrated the strong sensitivity of complex electrical resistivity to iron sulfide mineral transformations associated with the alternating redox conditions during microbial biomineralization and during oxidation of mine waste tailings (107-108). Placencia and Slater (109) fitted a mechanistic, electrochemistry-based model to complex electrical resistivity data acquired during the oxidation of pyrite and pyrrhotite.
1.4.2 Laboratory Methods

Laboratory approaches to measure contaminant transformation rates typically rely on microcosms. For fractured bedrock aquifers, rock samples from the site are often crushed and homogenized before they are used to construct microcosms. Darlington et al. (63) used these types of microcosms to study biotic and abiotic degradation pathways for TCE and cDCE in a fractured bedrock aquifer. First-order abiotic transformation rates of cDCE were obtained based on headspace monitoring results using a gas chromatograph with a flame ionization detector. However, it is unclear how crushing the rock may have impacted the rate and capacity for transformation, since crushing may alter the microbiology, geochemistry and pore structures of the rock.

More complicated laboratory-scale systems have been constructed to simulate dual porosity media. Doner (110) built a sand tank (0.84 m × 1.07 m) with four suspended, low permeability clay layers composed of sodium bentonite (Fig. 1-3). Tap water containing two tracers, fluorescein and bromide, was pushed through the tank for 22 days to simulate a loading period, followed by 100 days of water flushing without fluorescein or tracer to evaluate the back diffusion. Another experiment by Sale et al. (111) used tanks containing five vertical sand layers bounded by four silt layers, all 5 cm thick (Fig. 1-4). PCE or TCE and bromide tracer were added and six different treatments involving biological, chemical and physical processes were evaluated. Though these experiments took into consideration the effect of back diffusion, the use of unconsolidated material (sand and silt) made it inappropriate to evaluate fractured bedrock, because the porosity, pore size, and permeability are significantly different.
Figure 1-3. Photograph of a dual tracer, dual porosity sand tank study (110, 112).
Figure 1-4. Photographs of a multiple layer sand tank experiment (111).
Experimental systems that employ intact pieces of rock should generate more representative results than the use of crushed rock. However, due to the difficulty of constructing and monitoring such experiments, few have been conducted.

Schaefer et al. (113-114) employed discretely fractured sandstone blocks to evaluate residual DNAPL PCE dissolution processes and the effectiveness of bioaugmentation. Sandstone blocks (29 × 29 × 5 cm) were tapped by a chisel along a natural mineral bedding plane to induce a fracture, causing the rock to split. The irregular fracture surface was intended to mimic the naturally occurring bedding planes. The two half pieces were sealed with epoxy, and residual PCE was attained by injecting PCE in the fractures followed by a water chase. Artificial groundwater was then pumped through one edge of the fracture to the other end (Figure 1-5). The experiments suggested that the dissolution rate of PCE in the rock fracture may be substantially slower than in unconsolidated sands, due to smaller effective interfacial area to volume ratio of DNAPL in rock fractures than in sands. A *Dehalococcoides*-containing consortium (SDC-9) was used for bioaugmentation, which achieved significant PCE dechlorination and enhanced PCE dissolution.

More recent studies conducted by Schaefer et al. (115-116) designed a diffusion cell with minimally disturbed sedimentary rocks to evaluate the coupled diffusion and abiotic reaction of TCE under anaerobic conditions. The cell was built with a rock disk about 0.5 to 2 cm thick, mounted over a cut opening in a thin sheet of aluminum, and sandwiched between two stainless steel chambers (Fig. 1-6). Both chambers were filled with synthetic groundwater; one served as the source and was amended with TCE and
Figure 1-5. Discretely fractured sandstone blocks experiment, designed by Schaefer et al. (113-114).
Figure 1-6. Diffusion cell used by Schaefer et al. (115-116) to measure coupled diffusion and abiotic transformation through the cut rock sections.
tracer (iodide); the other served as the sink and did not contain TCE or tracer. Abiotic transformation of TCE was confirmed based on the production of acetylene, ethene, and ethane, with rates ranging from 8.3E-10 to 4.2E-8 s\(^{-1}\).

Chen et al. (117) developed an apparatus with an intact core of sandstone to evaluate fluid, heat, and transport of 1,2-dichloroethane during boiling and fracture depressurization. The apparatus consisted of a cylindrical sandstone core (5.08×30.48 cm) to represent the rock matrix, with one end of the core mimicking the fracture surface adjacent to the matrix (Fig. 1-7). The core was encased in heat shrinkable Teflon tubing and placed inside a pressure vessel so that contaminated water could be forced through the core to achieve a uniform distribution of 1,2-dichloroethane. The experimental data were fit with a numerical model. The results demonstrated the potential for using thermal remediation to remove VOCs from contaminated rock.

A similar experiment was constructed by Liu et al. (118), who used contaminated clay instead of intact rock. The clay was packed inside two types of experimental cells, one being a rigid-wall stainless steel tube and the other a flexible-wall Teflon tube in a pressurized chamber (Fig. 1-8).
Figure 1-7. Apparatus designed by Chen et al. (117).
Figure 1-8. Apparatus designed by Liu et al. (118).
1.4.3 Model Simulation

Different conceptualizations have been used to describe the flow system within fractured bedrock aquifers, treating them either as an equivalent porous continuum, a discrete fracture network, or dual porosity media (119). Simulation of groundwater flow and solute transport with reaction are generally achieved with numerical or analytical models, or something in between.

Numerical models solve differential equations using numerical approximations, such as finite differences and finite elements. Compared to analytical models, numerical models are mathematically simpler, more versatile, and better suited for computation. One of the well-known computer codes for groundwater modeling is MODFLOW, developed by the US Geological Service (120). Other popular numerical modules include HydroGeoSphere, FEFLOW, FRACTRAN, and COMSOL. One potential drawback with numerical models is that accurate simulation requires extremely high spatial and temporal discretization to capture the system geometry and concentration gradients (16). For example, Chapman et al. (112) used HydroGeoSphere, FEFLOW, and MODFLOW/MT3D to simulate the monitoring results from a bench scale sand tank. To accurately simulate matrix diffusion, a fine element mesh containing up to ~25,000 nodes was used for a 2D domain of 1.1×0.84 m.

Analytical models simulate contaminant transport assuming a simple uniform groundwater flow field and are therefore easier to set up and run. However, because analytical models do not consider complicated flow fields and transient flow effects, they are most appropriate for simple sites and screening-level evaluations. One example is
REMChlor (Remediation Evaluation Model for Chlorinated Solvent), developed by Falta (121). REMChlor is incapable of modeling fractured bedrock aquifers; however, an enhanced version (REMChlor-MD) is scheduled for release in 2017 and is capable of simulating matrix diffusion using a semi-analytical method. The principles, described in Falta and Wang (122), use a semi-analytical approximation for transient matrix diffusion in the low permeability zone, and generate matrix diffusion fluxes as source/sink terms for the numerical simulators. The calculation is more efficient than traditional fine-grid numerical simulation because the low permeability zones can be represented by coarse grids with analytical or numerical approximations made at the sub-gridblock scale.

The “Matrix Diffusion Toolkit” developed for the Department of Defense ESTCP program evaluates the effect of matrix diffusion. Major modeling tools in this toolkit are the Square Root Model and Dandy-Sale Model (123). However, this toolkit basically assumes two zones with different transmissivities, so that application to fractured bedrock aquifer requires additional interpretation and expertise.

1.4.4 Integration of Evidence

A summary of various methods to predict rates of contaminant transformation within low permeability rock is provided in Figure 1-9. Certain evidence may be stronger than the others. It is always good practice to look for consistency and correlation between different lines of evidence before drawing final conclusions. This is particularly important when estimating rates, which are essential inputs to models.

Field data often come first in the evaluation of a particular site. Unfortunately, in fractured bedrock, historical field data alone does not provide sufficient evidence of
degradation due to the occurrence of multiple attenuation processes. Geophysical characterization, geochemical modeling and CSIA can be supportive but are not suitable by themselves for accurate rate estimation.

Crushed rock microcosms constructed with material from the fractured bedrock site can provide unambiguous results based on their controlled nature. Short term studies (e.g., 30-40 days) serve as a useful tool to identify dominant degradation process and to evaluate the short term responses of amendment addition. However, slow rate phenomenon may not be detectable in this timeframe, such as mineral-mediated abiotic transformations, adsorption-desorption equilibrium, and biological degradation associated with relatively long lag times. Therefore, it may be necessary to monitor longer term crushed rock microcosms (e.g., 6-18 months) to generate a robust trend. This is particularly meaningful for predicting attenuation rates and transformation capacities. One issue of concern, however, is the uncertainty introduced by crushing the rock.

Intact rock core microcosms offer the potential to fill this methodology gap and produce more representative estimates of in situ degradation rates. However, intact core microcosms have not been widely used due to their more complex construction and operation. One of the objectives for this study was to develop a prototype that can be relatively easily built and maintained, and can be adapted to different types of low permeability media.
Figure 1-9. Methodologies for documentation of attenuation processes in fractured bedrock aquifers.
Similar to field data, a non-homogenous intact rock core microcosm requires advanced data interpretation to predict individual attenuation processes. Just as plume-scale models can be used to predict fracture flow and plume migration over decades to centuries of time, a micro-scale model can be developed to describe the behavior of a single rock core microcosm and predict in situ transformation rates that can be used in plume scale models.

1.5. Site Background

The rock and groundwater samples that were used for the research presented in this dissertation were obtained from an industrial site in southern California. It was formerly used mainly as a test center for rocket engines during the mid-twentieth century.

1.5.1 Geology

The site covers an area of 2,850-acres and is underlain with a unit referred to as the Chatsworth formation, consisting of highly fractured interbedded sandstone and shale deposited by marine turbidities that were uplifted during the Upper Cretaceous. Groundwater flow at the site is complex due to multiple faults, fractures, and variable groundwater recharge. The majority of groundwater flow occurs in the fracture network, with hydraulic conductivities (1E-5 to 7E-4 cm/s) much higher than that of the rock matrix (4.1E-7 cm/s). However, most of the groundwater resides in the porous rock body because matrix porosity (~13%) is almost four orders of magnitude larger than the bulk fracture porosity (i.e. the total void space provided by interconnected fractures within a unit aquifer volume) (124).
1.5.2 Contamination and Cleanup

TCE was used to degrease rocket engines and then disposed of in unlined evaporation ponds. Significant amounts of TCE soaked into the ground and reached the underlying aquifer. The majority of releases happened between the early 1950s and late 1960s, and was completely terminated in the 1990s. TCE percolated into the aquifer to depths in excess of 244 m. TCE levels up to 5,200 μg/L have been detected in the groundwater. The total amount of TCE released was estimated to be over 300,000 gallons (125).

Cleanup work started in the 2000s and included the dismantling of more than 300 structures, installation of 260 monitoring and extraction wells, analysis of over 38,000 soil and groundwater samples, and construction of groundwater treatment systems capable of removing TCE and cDCE (126). Parallel to the cleanup work were year-long studies of in situ chemical oxidation, vapor extraction, and laboratory evaluation of bioremediation (127).

1.5.3 Field Studies

Multiple lines of field evidence have been gathered to evaluate the main processes involved in attenuation of chlorinated ethenes at this site, including long-term volatile organic compound (VOC) data from monitoring wells, high resolution VOC characterization of the rock matrix, CSIA, and geochemical parameters (18). Extensive sampling for groundwater and cores has been done over a depth of 6 to 426 m since 1985. Groundwater was originally collected using the conventional three-volume purge method; in 2008, the depth discrete SNAP sampler method was adopted to improve the
detection limit and location accuracy. Analysis of groundwater samples has included VOCs (TCE and its diagnostic daughter products), CSIA (for TCE and cDCE), and hydrogeochemistry.

Compared to groundwater samples, which are most representative of flow in active fractures, rock core samples have been taken from within the low permeability zone away from fractures and were therefore more indicative of conditions within the matrix. Rock core samples were analyzed for mineralogy, TCE, cDCE, tDCE and 1,1-DCE. However, it has not been feasible to measure dissolved gases (e.g., ethene and acetylene) or perform CSIA in water associated with the rock samples, so assessment of complete dechlorination in the rock matrix has been lacking. (18)

Temporal analysis of quarterly groundwater VOC data over the past 25 years suggests a site-wide decrease in dissolved TCE levels. Compared to decreases in TCE as high as four orders of magnitude, decreases in cDCE have been lower. Nevertheless, given the complex geological setting, additional lines of evidence are needed for the occurrence of TCE and cDCE degradation, especially within the rock matrix, where most of the mass resides (18).

VOC analysis of both groundwater and rock samples indicates that cDCE is the dominant product of TCE reductive dechlorination. Minor levels of tDCE, VC and ethene have been detected, indicating that dechlorination beyond cDCE has occurred, but to a limited extent. Detection of acetylene, ethane, and 1,1-DCE suggests that TCE and/or cDCE are also undergoing abiotic transformation.
Sandstone from the site is abundant in iron-bearing minerals (i.e., iron sulfides (FeS), pyrite (FeS₂), fougerite, magnetite, biotite, and vermiculite) and solid-phase organic carbon (0.53-0.83%) (128). Average groundwater conditions for the site include a pH of 7.1, dissolved organic carbon at 2.1 mg/L, and redox levels that are predominantly anaerobic (128-129). These factors suggest that geochemical conditions may be conducive for transformation of chlorinated ethenes by biotic and abiotic processes.

δ¹³C measurements were performed with groundwater samples from the site (18). Values for TCE DNAPL could not be obtained, however, δ¹³C values for dissolved TCE in a potential source zone (-31.9 to -27.4 ‰) are similar to that of manufactured TCE. δ¹³C signatures for TCE across the site range from -30.5 to -11.8 ‰, although the levels are highly spatially variable. In general, TCE at the site is more ¹³C enriched than manufactured TCE, which is indicative of TCE degradation. δ¹³C values of cDCE range from -27.1 to -20.8 ‰ and tend to be more depleted than the δ¹³C values for TCE present at the same location, suggesting that limited further degradation of cDCE has occurred.

Parker et al. (130-131) developed a generic conceptual model that includes the dissolution of TCE DNAPL in the fractures and subsequent diffusion of dissolved TCE into the rock matrix. A 2D discrete fracture network transport model, such as FRACTRAN and HydroGeoSphere, was then used to simulate steady-state groundwater flow and transient TCE transport and degradation with site-derived parameters (17-18). These model simulations demonstrate a strong bulk retardation effect caused by a combination of diffusion, dispersion, sorption and degradation processes, in spite of rapid groundwater velocities in the fractures. The simulations show strong plume retardation.
due to matrix diffusion in the early contamination stage (<50 years) and a dramatic impact on plume behavior by different biotic and/or biotic degradation rates in later stages (>50 years). These simulations suggest that an extremely slow rate of degradation, equivalent to a half-life of 20 years, may be sufficient for plume attenuation over a long period.

1.5.4 Laboratory Studies

Using crushed rock and groundwater from the site, Darlington (129) constructed live and autoclaved microcosms to evaluate natural attenuation. Robust TCE reduction to cDCE was observed in live bottles but without significant further reductive dechlorination, consistent with only trace levels of VC and ethene detected in field samples. $^{14}$C-labeled TCE and cDCE were used to track non-specific daughter products from abiotic transformation. CO$_2$ was the predominant abiotic end-product in live microcosms, while CO$_2$ and soluble products accumulated in autoclaved bottles. Darlington (129) speculated that iron-bearing minerals in the Chatsworth sandstone mediated the abiotic transformation of cDCE and TCE (Fig. 1-2). However, the extent of transformation in microcosms was limited to approximately 25% of the cDCE.

1.6. Summary and Objectives

While reductive dechlorination of TCE to cDCE is well established at the industrial site, considerable amounts of TCE and cDCE still remain. This may be a consequence of insufficient reducing capacity, either in the form of electron donor for biotic reduction or reduced minerals in the sandstone for abiotic reduction.
The occurrence of reductive dechlorination of TCE to cDCE in unamended microcosms has been demonstrated (63). However, the fact that crushed rock was used should not be overlooked. Such microcosms may skew the evaluation, because 1) the ratio of sandstone to groundwater used (0.4 g/g) was much smaller than the ratio that exists in situ, causing a decrease in transformation capacity; 2) crushing increased the surface area of the sandstone, likely affecting the extent and rate of reaction; and 3) potential heating and air exposure during crushing may alter the mineralogy and affect the transformation capacity of the rock.

Further dechlorination of cDCE appears to be limited. Three possible explanations include: 1) Measurements of reduction-oxidation conditions indicate that redox potentials are generally positive from the phreatic surface to an approximate depth of 20 m, then become increasingly negative with depth (Fig. 1-10). Although the amount of naturally occurring biodegradable organics appears to be sufficient to support TCE reduction to cDCE in some locations, it is not sufficient to create the lower redox environment generally associated with high rates of cDCE reductive dechlorination (i.e., <-100 mV) (132); 2) Field evidence also indicates the presence of localized zones of iron-reduction, sulfate-reduction, and methanogenesis (128-129); these process can compete with halorespiration for the electron donor needed to sustain reductive dechlorination; and 3) It is likely that the microbial population is limiting the rate of cDCE reduction to ethene, i.e., *Dehalococcoides* and possibly *Dehalogenimonas* are either lacking in the subsurface of this site, present in low numbers, and/or heterogeneously distributed. Since these microbes thrive best under low redox potentials, the absence of activity is expected.
Figure 1-10. Site measurements of average ORP and representative redox changes with depth; courtesy of R. Andrachek.
Biostimulation is considered a promising remedy for this site, with potential benefits including the development of a dechlorinating biofilm and stimulation of bacteria in the rock matrix (section 1.2). In addition, abiotic transformation of chlorinated ethenes can be facilitated by lowering the redox potential and enhancing production of reactive minerals (section 1.3.3).

While crushed rock microcosms are an important tool for use in laboratory studies, crushing the rock may affect contaminant degradation rates. The need for an experimental system that leaves the rock intact is evident. Furthermore, advances in modeling should facilitate characterization of the intertwined processes that dominate in non-homogenous media, including matrix diffusion.

The overall objective of this dissertation was to evaluate the application of natural attenuation and biostimulation for remediation of TCE and cDCE in a fractured bedrock aquifer. The specific objectives were:

1) To evaluate the potential of using biostimulation to increase the rate of biotic and abiotic transformation of TCE and cDCE in fractured sandstone. This objective is addressed in Chapter 2 using crushed rock microcosms.

2) To evaluate the effect of biostimulation on remediation of TCE in intact cores, and study the interaction between the diffusion and attenuation processes. This objective is addressed in Chapter 3 using intact rock core microcosms.

3) To conduct numerical simulation of intact rock core microcosms to study diffusion and degradation processes and evaluate site-specific parameters. This objective is addressed in Chapter 4.
2. REMEDIATION OF CHLORINATED ETHENES IN FRACTURED SANDSTONE WITH NATURAL ATTENUATION AND BIOSTIMULATION: CRUSHED ROCK MICROCOSEM STUDY

2.1. Abstract

Biostimulation has been identified as a potential technology to treat a fractured sandstone aquifer contaminated by trichloroethene (TCE). The objective of this study is to evaluate the effect of biostimulation on reductive dechlorination and assess other degradation pathways at this site. $^{14}$C-labeled TCE and cDCE were used in crushed rock microcosms to quantify the rate and extent of product formation; enrichment in $\delta^{13}$C was measured in microcosms without $^{14}$C added to evaluate degradation.

Lactate, hydrogen release compound® (HRC), and emulsified vegetable oil (EVO) significantly increased the rate of TCE reduction to cDCE. Lactate also stimulated reductive dechlorination of cDCE to vinyl chloride (VC) and ethene, suggesting the presence of indigenous *Dehalococcoides* that are inactive due to donor-limited conditions. Illumina sequencing and qPCR analysis suggest that *Geobacter* spp. are responsible for reductive dechlorination of TCE to cDCE and *Dehalococcoides* spp. for reduction of cDCE to VC and ethene. The rate of VC reduction to ethene was much slower than for TCE to cDCE and cDCE to VC, suggesting that *Dehalococcoides* perform the final dechlorination step co-metabolically. This was subsequently confirmed in enrichment cultures fed with VC where no activity was observed. Abiotic transformation of TCE and cDCE was observed based on accumulation of $^{14}$C daughter
products and δ\(^{13}\)C enrichment in the absence of reductive dechlorination. Electron donor and sulfate amendments did not enhance abiotic transformation, but they did stimulate sulfate reduction. Rates of \(^{14}\)CO\(_2\) and \(^{14}\)C-soluble product accumulation in unamended microcosms were used to determine pseudo-first-order rates of abiotic transformation, which were 0.038 yr\(^{-1}\) for TCE and 0.044 yr\(^{-1}\) for cDCE, corresponding to half-lives of 14 yr for TCE and 16 yr for cDCE.

2.2. Introduction

Fractured bedrock aquifers tend to be more recalcitrant to active remediation compared to unconsolidated aquifers due to their complex flow systems and the presence of low permeability matrix. Monitored natural attenuation (MNA) and biostimulation are potentially favorable treatment methods due their cost effectiveness.

Natural attenuation of chlorinated ethenes in an anaerobic environment may occur through biological and abiotic pathways, including reductive dechlorination (i.e., hydrogenolysis), β-elimination, α-elimination, and hydrogenation (19-20) (Fig. 1-1). Reductive dechlorination is typically the dominant biological pathway. Two types of microbes are capable of reductively dechlorinating trichloroethene (TCE); one reduces TCE only as far as \(\text{cis-1,2-dichloroethene (cDCE)}\) (26, 32-47), while the other dechlorinates TCE to vinyl chloride (VC) and ethene. \textit{Dehalococcoides} is the predominant genus associated with reductive dechlorination to ethene (24-26, 28-29, 133), with more recent attention focusing on \textit{Dehalogenimonas} (30-31). Reductive dechlorination may be affected by unique features of fractured bedrock aquifers. Most of the contaminant mass may reside within the rock matrix as a consequence of diffusion;
this may reduce the prospects for contact with microbes, in rocks with pores smaller than microbial cell sizes (48). However, their presence in the rock should not be ruled out; several studies have detected microorganisms capable of TCE degradation within clay layers (51) and fractured sandstone-dolostone (52). Other factors affecting reductive dechlorination are potentially unfavorable redox conditions; the substrate needed for reductive dechlorination may be absent or distributed in isolated zones separated by elevated redox potentials; sulfate-reducing conditions may compete for electron donor, especially hydrogen.

Abiotic transformation of chlorinated ethenes may also contribute to natural attenuation. Documentation of abiotic transformation in fractured bedrock sites faces several challenges: 1) Loss of contaminants alone may be insufficient evidence due to the potential coexistence of other attenuation mechanisms, e.g., biodegradation, diffusion, adsorption, and dilution; 2) Acetylene is an unambiguous indicator of abiotic degradation of chlorinated ethenes (56), but is hard to track due to its high volatility, biodegradability, and lack of representative sampling techniques (59-60); 3) Abiotic daughter products such as CO₂ and organic acids (primarily glycolate, formate, and acetate) are difficult to detect, and their presence may be from reactions other than chlorinated ethene transformation (63, 134); 4) Geochemical and geophysical measurements (e.g., mineralogy and magnetic susceptibility) may be indicative of abiotic transformation mediated by iron-bearing minerals; however, iron-bearing minerals could be present at concentrations too low to be detected, yet they still could make a significant contribution to abiotic processes; and 5) Use of compound specific isotope analysis (CSIA) to
document abiotic degradation is complicated by the co-occurrence of biotic degradation processes, although advances in multiple isotope analyses are helping to sort this out. Additional evidence for abiotic degradation may be obtained through microcosm studies in which biotic activity can be eliminated and $^{14}$C-labeled contaminants can be used.

Biostimulation may enhance both biotic and abiotic transformation. Substrate addition generates redox conditions that are sufficiently low for reductive dechlorination, and fermentation yields hydrogen, a universal electron donor for chlororespiration. Acetate is also produced and used as the carbon source for *Dehalococcoides*. Biostimulation may also enhance abiotic transformation and interactions of biotic and abiotic processes that benefit overall remediation (16, 20, 93-96). Substrate addition includes the use of slowly fermentable compounds as well as more frequent addition of soluble compounds that are more rapidly fermented (135).

An industrial site in southern California is contaminated with TCE to depths in excess of 244 m. The majority of dissolved TCE diffused into or absorbed to the rock matrix. Field (128, 136) and laboratory evidence (63) strongly supports that reductive dechlorination to cDCE is a major attenuation process for TCE, however, further reduction to VC and ethene appears to be limited, potentially because of 1) lack of electron donor to create the reduced environment generally associated with high rates of cDCE reductive dechlorination (132); 2) lack of sufficient microbes that are capable of reducing cDCE to ethene; and 3) a high background level of sulfate that competes with reductive dechlorination for electron donors (137). Alternative degradation processes, predominantly abiotic transformation mediated by iron-containing minerals in the
sandstone, were detected by tracking specific daughter products using $^{14}$C-labeled TCE and cDCE (137). The extent of transformation reached 25% of the cDCE, indicating a potentially limited transformation capacity for the minerals or the presence of other inhibitory factors. Biostimulation was identified as a promising technology for this site.

The objectives of this study were to evaluate the potential for biostimulation to increase the rate of biotic and abiotic transformation of TCE and cDCE in fractured sandstone. Crushed rock microcosms were prepared with and without $^{14}$C-labeled TCE and cDCE. This permitted quantification of $^{14}$C-labeled product formation and quantification of $\delta^{13}$C enrichment.

2.3. **Material and Methods**

2.3.1 *Site Geology and Sample Collection*

The site is underlain by a geological unit referred to as the Chatsworth formation, consisting of highly fractured interbedded sandstone and shale deposited by marine turbidities that were uplifted during the Upper Cretaceous. Groundwater flow at this site is complex due to multiple faults, fractures, dips and groundwater recharges. The majority of groundwater flow occurs in the fracture network with much higher hydraulic conductivity (1E-5 to 7E-4 cm/s) than in the rock matrix (4.1E-7 cm/s). Most of the groundwater resides in the porous rock body, because matrix porosity (13.6%) is almost four orders of magnitude larger than the fracture porosity (124).

Sandstone was collected from a corehole at the site, crushed onsite and shipped to Clemson University. Groundwater was collected from two monitoring wells, one in the
source zone and one further downgradient. Detailed information about the sampling locations and collection methods are provided in Appendix A.1.

2.3.2 Chemicals and Medium

The following chemicals (purity, source) were used: tetrachloroethene (PCE; 99%, Acros Organics), TCE (99%, Alfa Aesar), $^{14}$C-TCE (99%, specific activity = 2 mCi/mmol, uniformly labeled, American Radiolabeled Chemicals, Inc.), cDCE (99%, TCI America), $^{14}$C-cDCE (99.1%, specific activity = 5 mCi/mmol, uniformly labeled, Moravek Biochemicals), VC (99.5%, Fluka), polymer grade ethene (99.9%, Airgas), ethane (99.95%, Matheson), methane (99%, Matheson), hydrogen (99.995%, Airgas), oxygen (99.8%, Airgas), and acetylene (99%, Matheson). Sodium lactate syrup was obtained from EM Science (58.8 to 61.2% sodium lactate; specific gravity 1.31). NewmanZone® emulsified vegetable oil (EVO) was obtained from RNAS. Hydrogen release compound (HRC®) was obtained from Regenesis. All other chemicals were reagent grade. An anaerobic mineral salts medium (MSM) for growing enrichment cultures was prepared as previously described (138) (Appendix A.2).

2.3.3 Crushed Rock Microcosms

Table 2-1 summarizes the experimental design, including five control treatments: water controls (WC), autoclave controls (AC), autoclave controls amended with sulfide (AS), unamended live (UN), and unamended live with sulfate added (S). Donor amended treatments included lactate (L), lactate plus sulfate (LS), EVO (E), EVO plus sulfate (ES), HRC (H), and HRC plus sulfate (HS). With 11 treatments, two compounds, and
Table 2-1. Summary of the different treatments for crushed rock microcosms. Each treatment included two sub-groups, i.e., 24 bottles with TCE as the contaminant (half with $^{14}$C-TCE added) and 24 bottles with cDCE as the contaminant (half with $^{14}$C-cDCE added). Numbers represent the number of the bottles out of 24 in each group that exhibited the indicated reductive dechlorination activity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCE Added</th>
<th>cDCE Added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no reductive dechlorination</td>
<td>TCE to cDCE (slow$^a$)</td>
</tr>
<tr>
<td>Water control (WC)</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Autoclaved control (AC)</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Autoclaved control + sulfide (AS)</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Unamended control (UN)</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Unamended control + sulfate (S)</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Lactate (L)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lactate + Sulfate (LS)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Emulsified vegetable oil, EVO (E)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>EVO + Sulfate (ES)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hydrogen release compound, HRC (H)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HRC + Sulfate (HS)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Slow = < 0.055 µM/d, fast = > 0.055 µM/d; rate based in the average amount of TCE reduced to cDCE divided by the time for the reaction to occur.
microcosms with and without $^{14}$C-TCE and $^{14}$C-cDCE, a grand total of 528 bottles were prepared. Microcosms were constructed in an anaerobic chamber as described by Darlington et al. (137), with minor modifications. Each 160 mL serum bottle contained 20 g of crushed rock and 50 mL of groundwater. Water controls were prepared with 50 mL of distilled deionized water plus an equivalent volume of glass beads as the crushed rock. The initial concentration of TCE and cDCE was ~1 mg/L. For bottles that received $^{14}$C-labeled TCE or cDCE, the $^{14}$C added was approximately 0.5 μCi/bottle. Details regarding bottle preparation are presented in Appendix A.3.

After time zero measurements, the bottles were returned to the anaerobic chamber for storage, and removed periodically for headspace and liquid sampling. Electron donors were initially added monthly; the frequency of addition was gradually decreased to every three or four months. Sulfide addition started on a weekly basis and was gradually reduced to every three months. Sulfate additions were made every three to four months. VOCs were analyzed weekly at the beginning; over time, the frequency was reduced to bi-weekly, and then monthly or longer. $^{14}$C analysis was carried out every three to four months. $\delta^{13}$C analysis was carried out on three cDCE treatments without $^{14}$C-cDCE.

2.3.4 Enrichment Cultures

Enrichment cultures were developed to evaluate the types of dechlorinating microbes present in microcosms that exhibited reductive dechlorination activity. Initially, transfers were made from the microcosms into groundwater (1.25%, v/v). Treatments were prepared with TCE as the electron acceptor and lactate as the electron donor. Further enrichment was done in anaerobic MSM (5% transfers, v/v), prepared with PCE,
TCE, cDCE, or VC as the potential electron acceptor and lactate as the electron donor, using a similar approach as described by Duhamel et al. (139). This provided information on the capability of the dechlorinating microbes to use the different chlorinated ethenes.

2.3.5 Analytical Methods and Determination of $^{14}$C Distribution

The total amount of VOCs present in the microcosms (PCE, TCE, cDCE, VC, ethene, ethane, acetylene, and methane) was monitored by gas chromatograph (GC) analysis of 0.5 mL headspace samples using a column packed with 1% SP-1000 on 60/80 Carbopack-B (140). Chloride, bromide, and sulfate were measured by an ion chromatography (IC) on an AS9-HC anion exchange column (Dionex, 4 mm x 250 mm; 9 mM Na$_2$CO$_3$ eluant, 1 mL/min). Organic acids were analyzed on a high performance liquid chromatography (HPLC) system on an Aminex® HPX-87H ion exclusion column (300 mm x 7.8 mm; 0.01 N H$_2$SO$_4$, 0.6 mL/min) at 210 nm wavelength. The procedures used to calculate the total quantity and distribution of $^{14}$C are outlined in Darlington et al. (137). Details are presented in Appendix A.4.

2.3.6 CSIA

Samples were periodically collected for measurement of $\delta^{13}$C-cDCE. Two mL groundwater from selected microcosms was diluted in 20 mL crimp top vials or 40 mL volatile organic analysis vials with minimal headspace, stabilized with a few drops of concentrated HCl, sealed with Teflon-faced septa, and shipped overnight on ice to the University of Waterloo Environmental Isotope Laboratory.
2.3.7 Genetic Analysis

Upon completion of monitoring, DNA was extracted from aqueous samples or crushed rock from select microcosms or enrichment cultures, using the MOBIOM PowerSoil® DNA Isolation Kit or PowerMax® Soil DNA Isolation Kit, following the manufacturer’s protocol. *Dehalococcoides, bvcA, vcrA* and *tceA* were quantified using the TaqMan®-Probe-based qPCR method (Appendix A.5), adapted from Loffler and colleagues (141-142). For other bacteria, including *Dehalobacter, Desulfitobacterium, Sulfurospirillum, Desulfuromonas, Geobacter* and *Geobacter lovleyi*, SYBR® Green Dye-based qPCR was performed using a protocol adapted from previous studies (143-146); detection limits and protocol specifics are presented in Appendix A.5.

For Illumina 16S sequencing, DNA extracts were sent to the Clemson University Genomics Institute or the Department of Biological Sciences for 16S Metagenomics sequencing. Sample preparation details are described in Appendix A.5.

2.4. Results and Discussion

Reductive dechlorination of TCE to cDCE occurred in two thirds of the live unamended bottles, mostly at a slow rate (Table 2-1). Biostimulation with lactate, HRC, and EVO significantly improved the rate of TCE reduction to cDCE, suggesting that dechlorinating microbes at the industrial site are lacking electron donor. Only lactate enhanced reduction of cDCE to VC and ethene; addition of sulfate inhibited this process.

The bottles with $^{14}$C added were incubated for a maximum of approximately 15 months, during which triplicates were sacrificed on four occasions to determine the
distribution of $^{14}$C. Bottles that did not receive $^{14}$C were monitored for as long as 41 months.

2.4.1 VOC Results

TCE reduction occurred to different extents in the eleven treatments (Fig. 2-1a, Appendix A.6). Four types of behaviors were observed (Table 2-1), including no reductive dechlorination (Fig. 2-1b), TCE reduction to cDCE at a slow rate (Fig. 2-1c), TCE reduction to cDCE at a fast rate (Fig. 2-1d), and TCE reduction to cDCE and further to VC and ethene (Fig. 2-1e). Low levels of cDCE showed up in all microcosms at time zero, due to background cDCE in the groundwater.

The average rate of loss of TCE in bottles in which reductive dechlorination was minor or not detectable was lower than the rates reported for PCE and TCE in water controls employed by other studies (147-149) (Appendix A.6). Loss in water controls was the lowest among all treatments and was presumably due to diffusion through septa and absorption onto glass beads. Average losses in autoclaved controls and autoclaved controls with sulfide added were higher compared to the water control, potentially a result of abiotic transformation or adsorption on to crushed rock. Also, the extent of loss in AC was higher than AS, indicating that addition of sulfide did not enhance abiotic degradation of TCE. Average decreases in the autoclaved controls (i.e., AC and AS) were smaller than those in the live treatments (i.e., UN and S).

Addition of lactate had a substantial impact by further stimulating reductive dechlorination of cDCE to VC and ethene in 15 out of the 24 lactate-amended bottles (L) and 5 of the 24 lactate plus sulfate bottles (LS). This was not expected, since VC and
Figure 2-1. (a) Average concentration profile for TCE in microcosms with TCE or $^{14}$C-TCE, and (b-e) representative VOC results in individual bottles.
ethene are detected at comparatively low levels in the field (18). Reduction of cDCE to VC occurred at a rate comparable to the rate of TCE reduction to cDCE, while subsequent reduction of VC to ethene occurred at a much slower rate. This slow rate was presumably not a consequence of a lack of electron donor, since repeated additions of lactate were made over time, in considerable excess of the electron equivalents needed to complete the reduction. In 9 of the 24 bottles, methane started to accumulate after several years of incubation, indicative of an abundance of electron donor. Fewer bottles in the lactate + sulfate treatment (LS) showed reductive dechlorination than lactate-alone treatment, indicating that sulfate had an inhibitory effect. The reason that neither EVO nor HRC stimulated reduction of cDCE is possibly due to their slower fermentation rate to produce hydrogen, the required electron donor for *Dehalococcoides*.

Reduction of cDCE in the 11 treatments with cDCE added occurred to different extents (Fig. 2-2a, Appendix A.7). Two types of behaviors were observed: no reductive dechlorination (Fig. 2-2b), or cDCE reduction to VC and ethene (Fig. 2-2c). Reductive dechlorination occurred only in the lactate treatment (L; 16 out of 24 bottles) and the lactate plus sulfate treatment (LS; 3 out of 24 bottles). This is consistent with results in the TCE microcosms. However, the onset of cDCE reduction took longer than in TCE microcosms.

The average rates of decrease of cDCE concentration in microcosms that lacked reductive dechlorination were lower than the rates reported for water controls in other studies (147-149) (Appendix A.7). Concentration decreases in the water controls were presumably due to diffusion through septa and absorption onto glass beads. Greater
Figure 2-2. (a) Average concentration profile for cDCE in microcosms with cDCE or $^{14}$C-cDCE, and (b, c) representative VOC results for individual bottles.
decreases in cDCE in the other controls in this study were likely due to adsorption and/or reaction with the crushed rock. Concentration decreases for cDCE in controls were generally less than those for TCE, perhaps due to a slower diffusion rate or a greater extent of TCE adsorption.

Acetylene was detected in TCE and cDCE microcosms (Fig. 2-3, Appendix A.8). It never exceeded 9 mol% of the TCE or cDCE initially present in the microcosms and was generally below 3 mol%. This is consistent with the low levels of acetylene detected at the industrial site (18, 128). Acetylene typically does not accumulate to the same molar concentration as chlorinated ethenes that undergo abiotic transformation.

2.4.2 Sulfate and Organic Acids

The groundwater contained ~1.8 mM of sulfate. Four of the treatments (S, LS, ES, and HS) received additional sulfate. Average levels of sulfate added and consumed are shown in Figure 2-4 for microcosms without $^{14}$C added; results for bottles with $^{14}$C added were similar (Appendix A.9). Most of the sulfate added was consumed in the treatments with electron donor added, indicating the presence of sulfate reducing bacteria. There was no consumption of sulfate (present in the groundwater) in the autoclaved controls and autoclaved controls with sulfide added, and limited sulfate consumption in the live unamended controls, consistent with a lack of electron donor.

Organic acids were analyzed in selected bottles from all live treatments without $^{14}$C-TCE or cDCE added between days 420 and 480. To facilitate preparation of a mass balance, amounts per bottles were converted to units of chemical oxidation demand (COD). Average results are shown in Figure 2-5. The COD associated with sulfate
Figure 2-3. Acetylene production in (a) TCE microcosms without $^{14}$C-TCE added, and (b) cDCE microcosms without $^{14}$C-cDCE added. Percentage recovery was calculated assuming an initial TCE or cDCE level of 0.6 µmol/bottle.
**Figure 2-4.** Sulfate results for (a) TCE microcosms without $^{14}$C-TCE added and (b) cDCE microcosms without $^{14}$C-cDCE added. Each bar represents the average result for triplicate bottles from each treatment and duplicate samples for each bottle, with error bars representing the standard deviation.
Figure 2-5. COD balance for (a) live TCE microcosms without $^{14}$C-TCE added and (b) live cDCE microcosms without $^{14}$C-cDCE added. Each bar represents the average result for two HPLC analyses performed on days 420 and 480, on triplicate bottles from each treatment and duplicate samples from each bottle, with error bars representing the standard deviation. UNF = unidentified fraction; RD = COD used for reductive dechlorination.
reduction and reductive dechlorination is also shown, as well as the COD that was unaccounted for (UNF). Conversions are based on 12 meeq/mmol lactate, 8 meeq/mmol acetate, 10 meeq/mmol propionate, 8 meeq/mmol sulfate, and 2, 4, or 6 meeq/mmol TCE when it is reduced to cDCE, VC and ethene, respectively.

In the treatments with donor added, an overall average of 77±9% (α=0.05) of the COD added was accounted for as organic acids, plus sulfate reduction. Reductive dechlorination was a minor contributor to the COD mass balance, indicating that only a small percentage of the donor added was used for this purpose. In treatments with sulfate added, sulfate reduction was stimulated and consumed a large percentage of the COD. No significant level of organic acids was detected in the unamended microcosms, consistent with a lack of electron donor in the groundwater.

2.4.3 $^{14}$C distribution and rate calculations

$^{14}$C-TCE and $^{14}$C-cDCE was added to one half of the TCE and cDCE microcosms. The stock solutions used to prepare the microcosms contained approximately 3% ($^{14}$C-TCE) or 1% ($^{14}$C-cDCE) impurities based on measurements of $^{14}$CO$_2$ and $^{14}$C-NSR immediately after adding the stock solution to bottles containing distilled deionized water (DDI). These initial impurities need to be considered when evaluating transformation processes other than reductive dechlorination. However, based on previously reported $^{14}$CO$_2$ and $^{14}$C-NSR production from TCE and cDCE (up to 25%) in microcosms containing sandstone at this site (137), these background levels were considered acceptable and therefore no purification of the $^{14}$C stock solutions was performed.
Overall $^{14}$C-VOCs were consistent with the GC results, i.e., cDCE, VC and ethene were the predominant products (Appendix A.10). Accumulation of $^{14}$CO$_2$ and $^{14}$C-NSR (Fig. 2-6) was slower compared to reductive dechlorination. The rate of accumulation was statistically significant ($p<0.05$) for seven of the treatments with $^{14}$C-TCE added (UN, L, E, ES, H, HS, and AS) and five of the treatments with $^{14}$C-cDCE added (UN, S, LS, HS, and AC). Overall, live treatments had higher levels of $^{14}$CO$_2$ but lower levels of $^{14}$C-NSR compared to the water and autoclaved controls, suggesting that microbial activity mineralized some of the $^{14}$C-NSR into $^{14}$CO$_2$. Previous microcosms with material from the same site revealed the major components of NSR as acetate, formate and glycolate (63), which are substrates readily used by microbes. This may explain why $^{14}$C-NSR levels remained elevated when an electron donor was added (especially for $^{14}$C-TCE, Fig. 2-6a) as the donor provided the microbes with a preferable substrate.

Up to 6.4% of the radioactivity in water controls was recovered as $^{14}$CO$_2$ plus $^{14}$C-NSR in $^{14}$C-TCE. This was approximately twice as high as the amount initially present (~3%). Degradation of $^{14}$C-TCE in the water controls was likely a consequence of autoradiolysis, during which the energy emitted from the radioactive material decomposed water molecules and formed free radicals, which subsequently degraded the radioactive compound itself (150-151). The DDI water in the $^{14}$C-TCE water control has no compounds other than TCE that could quench the radicals when the experiment started. During this initial reaction, $^{14}$CO$_2$ and $^{14}$C-NSR was formed and quenched by accumulation of products, preventing further autoradiolysis beyond the first sampling event (~90 d). In contrast, autoradiolysis in other treatments with groundwater was likely...
Figure 2-6. Recovery ratio of $^{14}$CO$_2$ and $^{14}$C-NSR in (a) $^{14}$C-TCE and (b) $^{14}$C-cDCE microcosms, and first order transformation rates for (c) TCE and (d) cDCE determined based on accumulation of $^{14}$CO$_2$ + $^{14}$C-NSR in the unamended microcosms. Percentages in (a) were calculated based on the initial $^{14}$C-TCE or $^{14}$C-cDCE levels. Each bar represents the average of triplicate bottles; error bars represent the standard deviation. An asterisk (*) by the treatment name indicates that the rate of increase of $^{14}$CO$_2$ + $^{14}$C-NSR is statistically greater than zero ($p<0.05$). Asterisk(s) above a bar indicate that the sum of $^{14}$CO$_2$ + $^{14}$C-NSR is statistically greater than the corresponding WCs at the 90% (*) or 95% (**) confidence level (one-tail Student’s $t$-test).
prevented by the presence of carbonates and natural organic matter, which act as scavengers for free radicals (152). In $^{14}$C-cDCE water controls, $^{14}$CO$_2$ plus $^{14}$C-NSR remained close to the amount initially present (~1%), indicating that autoradiolysis is a minor process with this compound. Purification of the $^{14}$C-TCE stock solution or use of filter-sterilized groundwater controls to minimize autoradiolysis would likely have avoided this complication. Nevertheless, most of the microcosms ended up with $^{14}$CO$_2$ plus $^{14}$C-NSR levels that were statistically greater than in the water controls (indicated by one asterisk ($p<0.1$) or two ($p<0.05$) above the bars in Fig. 2-6, panels a and b).

$^{14}$C-cDCE autoclaved microcosms showed the greatest increase in $^{14}$CO$_2$ plus $^{14}$C-NSR among all treatments, reaching 10.3% by the final evaluation event (Fig. 2-6a). $^{14}$C-TCE autoclaved controls reached a plateau in $^{14}$CO$_2$ plus $^{14}$C-NSR of 7.5%-8.7% after the first evaluation event (~90 d; Fig. 2-6b). The plateau in transformation occurred in a previous microcosm study constructed with crushed rock from the same site; accumulation of $^{14}$CO$_2$ and $^{14}$C-NSR stopped within 2-3 months for $^{14}$C-TCE and 7-8 months for $^{14}$C-cDCE (63). The authors suspected that the transformation capacity of the sandstone was exhausted. However, throughout the incubation period of this study, no slowing down or plateau was observed for product accumulation in unamended and autoclaved $^{14}$C-cDCE bottles. It appears that the capacity was not yet reached in these bottles; slight variations in mineral composition for different batches of rock samples may have contributed to the variability. It may also indicate that other factors limit the transformation.
Sulfide added to autoclaved controls did not enhance abiotic transformation. On the contrary, the reductant appeared to inhibit transformation of $^{14}$C-cDCE to $^{14}$CO$_2$ and $^{14}$C-NSR, possibly related to conversion of mackinawite (FeS) to pyrite (FeS$_2$), which has a much lower dechlorinating rate (153-154).

Electron donors were added with and without sulfate. The intent of adding sulfate was to stimulate abiotic degradation via biologically generated sulfide. Although sulfate reducing bacteria were readily stimulated by repeat additions of electron donor and sulfate, production of $^{14}$CO$_2$ and $^{14}$C-NSR was not increased. Instead, addition of an electron donor somewhat diminished accumulation of $^{14}$CO$_2$ and $^{14}$C-NSR in the $^{14}$C-cDCE microcosms, in comparison to microcosms with less sulfate reduction. Similar to direct addition of sulfide, biologically generated sulfide may have decreased the reactivity of minerals involved in abiotic transformation of TCE and cDCE (153-154). It is less clear if reductive dechlorination of TCE to cDCE impacted abiotic transformation; it seems that production of $^{14}$C-VC slowed down or even decreased total $^{14}$CO$_2$ and $^{14}$C-NSR accumulation, as in the last three bottles sampled for $^{14}$C in the lactate amended treatments with $^{14}$C-TCE or $^{14}$C-cDCE, and in the lactate plus sulfate amended treatment with $^{14}$C-TCE (Fig. 2-6, panels a and b, Appendix A.10).

Since consistent increases in $^{14}$CO$_2$ plus $^{14}$C-NSR were observed in unamended microcosms where minimum reductive dechlorination occurred, they were used to determine pseudo-first order rate constants for transformation of TCE and cDCE by processes other than reductive dechlorination. Basing the rate constant on accumulation of $^{14}$C-daughter products is preferable to using only GC headspace measurements, as this
analysis does not take into account adsorption of TCE or cDCE to the crushed rock and therefore tends to overestimate the extent of transformation. Product formation was normalized to the amount of $^{14}$C initially added, as shown in Figure 2-6, panels c and d. The rate constant for TCE is $0.038 \pm 0.011 \text{ yr}^{-1}$ ($\alpha=0.05$), or a half-life of 18 yr (95% confidence interval of 14 to 25 yr), and the rate for cDCE is $0.044 \pm 0.022 \text{ yr}^{-1}$ ($\alpha=0.05$), or a half-life of 16 yr (95% confidence interval of 11 to 31 yr).

These rates are lower than the values determined by Darlington et al. (134) for crushed sandstone from this site, which were $8.7 \pm 2.1 \text{ yr}^{-1}$ in unamended microcosms and $5.4 \pm 1.1 \text{ yr}^{-1}$ in autoclaved controls (both $\alpha=0.05$). The fact that Darlington et al. used GC headspace results may result in overestimation of the rates, because the amount of cDCE adsorbed to the rock was not excluded from the total loss. In addition, high transformation rates for cDCE lasted only 20-30 days, after which the decrease in cDCE entered a plateau, consistent with the equilibrium process of adsorption. In this study, accumulation of $^{14}$CO$_2$ plus $^{14}$C-NSR continued for over 460 days, so the rate constants through this experiment should be more representative of field conditions.

2.4.4 $\delta^{13}$C Results

Analysis of $\delta^{13}$C was performed in samples from the water controls, autoclaved controls and unamended live microcosms without $^{14}$C-cDCE added (Fig. 2-7).

In the corresponding unamended and autoclaved microcosms with $^{14}$C-cDCE added, there were statistically significant increases in $^{14}$CO$_2$ plus $^{14}$C-NSR. In the unamended live microcosms, $\delta^{13}$C-cDCE increased from an average of $-25.0\%$ to $-22.0\%$, with an enrichment rate of $\sim0.008 \%$/day. In autoclaved controls, $\delta^{13}$C-cDCE
increased from an average of -24.5‰ to -21.0‰, with an enrichment rate of ~0.009‰/day. The slopes of the trend lines for the unamended and AC treatments are statistically significant ($p<0.05$) while the slope for the water controls is not ($p>0.05$). The lack of $\delta^{13}$C enrichment in the water controls suggests that diffusional loss of cDCE through the septa did not contribute to enrichment. Adsorption may have affected the magnitude of enrichment. Nevertheless, accumulation of $^{14}$CO$_2$ plus $^{14}$C-NSR in the unamended and autoclaved microcosms correlated with enrichment in $\delta^{13}$C, suggesting enrichment was a consequence of cDCE transformation via processes other than reductive dechlorination.

2.4.5 Enrichment Cultures

Enrichment cultures developed from the microcosms were prepared with PCE, TCE, cDCE, or VC as potential electron acceptors; representative results are shown in

Figure 2-7. Change in $\delta^{13}$C-cDCE in three cDCE microcosms without $^{14}$C-cDCE added.
Appendix A.12. TCE and cDCE were repeatedly dechlorinated at a high rate, with accumulation of VC, which then underwent a slower rate of dechlorination to ethene. In contrast, the enrichment that received only VC did not exhibit any reductive dechlorination. The enrichment that received PCE initially reduced PCE to cDCE but stopped after three additions of PCE were consumed; addition of TCE, a potential primary substrate for the dechlorinators, did not restore activity on PCE. These results suggest that the enrichment cultures contained microbes capable of respiring TCE and cDCE, but not PCE or VC. The activity on PCE and VC appeared to be cometabolic.

2.4.6 qPCR and Illumina Sequencing

qPCR results are presented in Figure 2-8a. *Dehalococcoides* only amplified in lactate-amended microcosms that exhibited cDCE reduction to VC and ethene, with concentrations ranging from 0.5-1.5×10⁹ copies/L. Levels in the unamended TCE and cDCE microcosms were below detection. Similar levels of *tceA* were detected, while *vcrA* and *bvcA* were below their detection limits. This suggests that *Dehalococcoides* spp. containing *tceA* were predominant. Such strains are known to respire TCE to cDCE and cDCE to VC; reduction of VC to ethene occurs cometabolically, at a slower rate. This is consistent with observations in the microcosms and enrichment cultures. The two *Dehalococcoides* strains known to possess the *tceA* gene are *D. mccartyi* strain 195 and *Dehalococcoides* strain FL2.

Lima et al. (52) evaluated samples from this site but did not detect any *Dehalococcoides*. This is likely a consequence of the low level of electron donor, i.e., a small population of *Dehalococcoides* is likely present but their numbers are too low to be
detected. Only after enhancing their growth in the microcosms by addition of lactate did their in situ presence become apparent.

qPCR analyses identified the *Geobacter* genus in all of the samples at $10^5$-$10^9$ copies/L, while *G. lovleyi* was only detected in lactate-amended bottles (Fig. 2-8b, Appendix A.13).

Illumina sequencing results were consistent with qPCR. *Dehalococcoides* spp. and *G. lovleyi* were only present in bottles that exhibited TCE and cDCE reduction to ethene, while the *Geobacter* genus was detected in all of the samples tested (Appendix A.13). The absence of *Dehalococcoides* spp. in microcosms that reduced TCE only to cDCE suggests that this first dechlorination step was mediated by a different type of microbe, e.g., *Geobacter*. The ubiquity of the *Geobacter* genus in the samples evaluated is consistent with their metabolic versatility and their ability to use ferric iron as an electron acceptor with a variety of electron donors. Species *G. lovleyi* is well known for its ability to reduce PCE and TCE to cDCE. However, *G. lovleyi* was not detected in the unamended bottles that exhibited TCE reduction to cDCE, implying a species of *Geobacter* that has not previously been shown to perform chlororespiration is present at this site. Detection of *G. lovleyi* in lactate-amended bottles indicates that lactate may have given *G. lovleyi* a kinetic advantage that favored their growth over that of other TCE respiring *Geobacter*.

Sequencing also revealed the presence of *Pelobacter acetylenicus* in several microcosms in which acetylene was detected, suggesting that *P. acetylenicus* may be responsible for consumption of acetylene formed via abiotic pathways (Appendix A.13).
Figure 2-8. Representative qPCR results for (a) *Dehalococcoides* 16S rRNA gene and reductive dehalogenase gene *tceA* (*verA* and *bvCA* levels were below the detection limit therefore no data were shown) and (b) the *Geobacter* 16s rRNA gene and *G. lovleyi*-specific 16S rRNA gene, analyzed with DNA extracted from groundwater in the microcosms. Error bars represent standard deviations. An asterisk after the treatment name indicates that all tested genes in the samples were below the limits of detection.
2.5. **Implications for Site Remediation**

While reductive dechlorination of TCE to less toxic cDCE is well established at the industrial site, considerable amounts of TCE still remain. Addition of lactate, EVO and HRC to crushed rock microcosms stimulated rapid and complete TCE reduction to cDCE, suggesting that in situ dechlorinators are starved for electron donor. There are several potential benefits associated with enhancing TCE reduction to cDCE in fractured bedrock aquifers. One is the rapid removal of TCE inside the fractures would speed up diffusion of TCE out of the matrix, making it available for other forms of treatment and thereby shortening the overall treatment time. It is also possible for a biofilm to develop over the fracture surface during biostimulation, which may treat the TCE diffusing out of the rock. Studies have shown that biofilms can decrease rock permeability (5-7); in that respect, the biofilm could be used as a containment technology. The ideal situation would be for biostimulation to activate indigenous bacteria inside the rock matrix where the majority of TCE mass resides. cDCE is less toxic and more mobile than TCE, suggesting it should diffuse out of the matrix at a faster rate. Intact rock core microcosms will provide a better test system to evaluate this expectation.

Lactate addition stimulated cDCE reduction to non-hazardous ethene, a promising end-point for in situ TCE and cDCE degradation. Transient accumulation of VC poses an elevated risk, since it is more toxic than the other chlorinated ethenes. However, VC reduction to ethene was rate limiting. Multiple lines of evidence point to the fact that VC was cometabolically degraded by *Dehalococcoides* sp., consistent with reduction of VC.
to ethene being the slowest step. Considering the heterogeneity of the fracture flow system, it may be difficult to control the migration of VC.

EVO and HRC did not stimulate reductive dechlorination of cDCE to VC and ethene, possibly because they fermented too slowly to generate sufficient hydrogen to support the growth of *Dehalococcoides*. This could be viewed as an advantage over lactate, since no undesirable VC was produced. Besides, use of lactate in fractured bedrock poses practical challenges, because it is subject to flushing by groundwater flow through the fracture network. EVO and HRC are less likely to be washed out and ferment more slowly, increasing the opportunity to develop a biofilm at the fracture surface. The applicability of biostimulation needs to be evaluated in more realistic experiments that simulate fracture flow and matrix diffusion.

Transformation of TCE and cDCE by pathways other than reductive dechlorination was identified via diagnostic ¹⁴C-labeled daughter products and δ¹³C-cDCE enrichment. Compared to a previous study in which the transformation capacity of the sandstone reached a plateau (63), ¹⁴CO₂ and ¹⁴C-NSR accumulated over time at a consistent rate. This enabled the calculation of pseudo-first order rate constants, which were 0.038 yr⁻¹ for TCE and 0.044 yr⁻¹ for cDCE, corresponding to half-lives of 14 yr for TCE and 16 yr for cDCE. Model simulations for this industrial site (17) suggest that a degradation half life of 20 years is sufficient to retard movement of the plume fronts and sustain mass reduction.

However, transformations rates derived from crushed rock microcosms need to be interpreted with caution because of the associated uncertainties. Transformation rates
were based only on $^{14}$CO$_2$ and $^{14}$C-NSR, so they are likely conservative. Other $^{14}$C products may have formed but were not detected with the methodology used. These rates may not be representative for the whole site. Different abiotic transformation patterns were observed in a previous study using crushed sandstone from the site (63), suggesting a variety of rock types are present and each may have different transformation capacities. The impacts of rock processing (i.e., crushing and homogenization) are poorly understood. For example, crushing may enhance the transformation rate by exposing imbedded minerals inside the rock.

In conclusion, biostimulation significantly enhanced TCE reduction to cDCE. Complete reduction of TCE to ethene is possible in response to electron donor addition, but with the considerable risk that there may be transient accumulation of VC. The abiotic-mediated component of natural attenuation appears to occur at a rate that stabilizes the plume and promotes ultimate mass reduction. However, there are uncertainties associated with the rate estimation, and further characterization using a more realistic system is warranted. A refined microcosm study using intact rock cores was developed to meet this research need, as described in Chapter 3.
3. REMEDIATION OF CHLORINATED ETHENES IN FRACTURED SANDSTONE WITH NATURAL ATTENUATION AND BIOSTIMULATION: INTACT CORE MICRO COSM STUDY

3.1. Abstract

A fractured sandstone aquifer at an industrial site in southern California is contaminated with trichloroethene (TCE) to depths in excess of 244 m. Identified natural attenuation processes include reductive dechlorination of TCE to \textit{cis}-1,2-dichloroethene (cDCE) and abiotic transformation of TCE and cDCE to acetylene, CO$_2$ and soluble compounds. A microcosm study using site groundwater and crushed sandstone demonstrated that addition of lactate may stimulate complete biotic reductive dechlorination of TCE to ethene, consistent with the detection of \textit{Dehalococcoides} genes. Abiotic transformation of TCE and cDCE was identified via product accumulation from $^{14}$C-TCE and $^{14}$C-cDCE, and from these results rates of transformation were determined. Enrichment in $\delta^{13}$C was also observed. Nevertheless, use of crushed rock creates uncertainties regarding how applicable the results are to field conditions with intact rock. The objective of this study was to evaluate natural attenuation and biostimulation using intact rock core microcosms, and to compare the results with those from crushed rock microcosms. Results from the intact core microcosms confirmed observations with crushed rock that biostimulation with lactate created reducing conditions and stimulated sulfate reduction as well as reductive dechlorination. However, reductive dechlorination stalled at cDCE, indicating a low population or absence of indigenous \textit{Dehalococcoides} in the specific rock cores evaluated. Enrichment in $\delta^{13}$C was observed for TCE in
microcosms that did not undergo a discernible level of reductive dechlorination, and for cDCE formed via reductive dechlorination of TCE. Enrichment suggests that alternative transformation pathways for TCE and cDCE previously observed in crushed rock microcosms also occurred in intact rock core microcosms. The intact core microcosms permitted evaluation of natural attenuation and biostimulation in realistic fractured bedrock, and by extension has applicability to evaluating remediation in other low permeability environments.

3.2. Introduction

Contaminant migration and plume behavior in fractured bedrock aquifers are commonly controlled by matrix diffusion in addition to advection and dispersion. Permeable fractures act as conduits for groundwater flow and the less permeable but high capacity matrix serves as a primary storage place for contaminants. This leads to several challenges for remediation.

Most treatments (except for thermal remediation and electro-osmosis) require delivery of soluble or suspended amendments to the contaminants. Therefore, the extent of amendment delivery is often restricted to the fractures where active groundwater flow occurs, and can be limited by small pore throat sizes in low permeability zones (16). Thorough characterization of groundwater flow and contaminant location is critical for remedies to be successful in fractured bedrock aquifers, but is challenging due to the intrinsic heterogeneity. Consequently, containment technologies, such as pump and treat, are often used to avoid the need to accurately locate the contaminant (1). However, within a reasonable remediation timeframe, pump and treat may only be effective in
removing contaminants from the fractures, while the majority of the mass tends to reside in the rock matrices. After the active treatment, back diffusion of contaminant from the rock matrices to the fractures may cause concentration rebound in the groundwater, and sustain a plume that lasts decades or even centuries (2-4, 16, 130-131, 155).

Natural attenuation in sedimentary rock is favored by the reducing conditions in the rock matrix, which often contain organic matter and reduced minerals. If attenuation occurs at a sufficient rate, the plume front may be retarded or even shrink. The long retention times within the matrix may ultimately lead to substantial mass reduction (16). The rate of contaminant degradation is one of the key criteria when assessing the effectiveness of monitored natural attenuation (MNA).

If MNA alone is not sufficient for site remediation, biostimulation may be used to enhance indigenous microbes. Studies have demonstrated the possibility of cultivating biofilm on the fracture surface as a barrier against contaminant migration (5-7). A time-release substrate that adsorbs to the sandstone may help sustain microbial activity. Furthermore, while the likelihood of microbes penetrating significantly into the rock matrix may be small, this possibility should not be ruled out (51-52). Furthermore, stimulation of biological processes may facilitate the transformation of cDCE and TCE via abiotic pathways, as frequently employed in BiRD (biogeochemical reductive dechlorination) or BMAD (biologically mediated abiotic degradation) techniques.

Evaluation of an in situ remedy often involves estimation of a degradation rate that is applicable to the rock matrix, which in the context of fractured bedrock may also have a major impact on the rate of back diffusion. Unlike unconsolidated aquifers, it is
not feasible to measure transformation rates within the rock matrix based on concentration changes over a distance. Rate determination in low permeability media usually involves bench-scale testing in microcosms, although more complicated systems such as flow-through columns or tanks have been used (16, 111-112). Rock samples are often crushed and homogenized to prepare microcosms. It is unclear how crushing affects the rate and extent of attenuation, because changes in structure may increase or decrease the rates. For example, crushing tends to open most of the pores and thereby increases contact among the contaminants, microbes, and mineral surfaces. In an undisturbed state, the pore throat size of sedimentary rock varies from 0.005 to 0.1 μm in shales, 0.03 to 2 μm for tight-gas sandstones, to over 2 μm in conventional reservoir rocks (48). The lower ranges may prevent the migration of microbes, which have diameters on the order of micrometers (49). Crushing reduces this limitation and may result in an overestimation of transformation rates.

Minerals such as magnetite and iron sulfides are commonly present in the rock matrix at very low concentrations, yet as little as 1% can make a significant contribution to abiotic processes. Because these minerals are vulnerable to oxidation, they may be compromised by extensive exposure to air during crushing. The crushing process may also alter the mineral structure and surface area in ways that increase or decrease the transformation rate relative to undisturbed rock.

Another drawback to crushing is that it removes the impact of matrix diffusion, a process highly dependent on the porosity and permeability of rock matrices (156). Porosity may be lower than 1% in igneous and metamorphic rocks or as high as 40% in
sandstone and limestone (157-158); permeability can vary from 1E-20 m² in granite to 1E-14 m² in sandstone (159). Crushing may change these properties and alter the impact of matrix diffusion on transformation rates.

Microcosms consisting of undisturbed rock provide a more realistic environment to evaluate transformation rates, but their application thus far has been limited. Schaefer et al. (113-114) employed discretely fractured sandstone blocks to evaluate dissolution of residual tetrachloroethene (PCE) DNAPL and the potential effect of bioaugmentation. PCE dissolved more slowly in rock fractures than in unconsolidated sand media, and bioaugmentation was effective in enhancing PCE dissolution and dechlorination. More recent studies by Schaefer et al. (115-116) used a diffusion cell with a minimally disturbed sedimentary rock to evaluate the coupled diffusion and abiotic reaction of TCE under anaerobic conditions. Transformation products were measured and abiotic reaction rates of 8.3E-10 s⁻¹ to 4.2E-08 s⁻¹ were determined. Chen et al. (117) designed an apparatus containing an intact sandstone core to evaluate thermal treatment and demonstrated good mass recovery. For intact core microcosms to be successful they must be leak proof, and they should minimize diffusive losses of volatile organic compounds (VOCs), which is particularly challenging as VOCs can diffuse through materials used to encase the rock (e.g., rubber, epoxy and Teflon). Most of the rock should be saturated with contaminated groundwater to replicate in situ conditions, and they must be operated in a manner that replicates contact with a fracture surface and allows for monitoring of changes in VOC concentrations.
In this study, intact rock core samples were collected from a fractured bedrock aquifer in southern California contaminated with TCE and *cis*-1,2-dichloroethene (cDCE). Field (128, 136) and laboratory evidence (63) indicate that reductive dechlorination of TCE to cDCE and abiotic transformation of TCE and cDCE to acetylene, CO₂ and soluble compounds are the most likely transformation pathways. Results from a microcosm study with crushed rock and groundwater from the site (Chapter 2) demonstrated that lactate is an effective electron donor for enhancing reductive dechlorination. The objective of this study was to evaluate transformation of TCE in intact rock core microcosms under conditions that simulate natural attenuation and biostimulation with lactate, and to compare the results to ones from crushed rock microcosms.

3.3. **Material and Methods**

3.3.1 *Site Geology and Sample Collection*

The site is underlain by the Chatsworth formation, a fractured interbedded sandstone and shale deposited by marine turbidities that were uplifted during the Upper Cretaceous. Groundwater flow at this site is affected by multiple faults, fractures, dips and groundwater recharges. The majority of groundwater flow occurs in the fracture network, which has an effective hydraulic conductivity (1E-7 to 7E-6 m/s) significantly higher than that of the rock matrix (4.1E-9 m/s). However, most of the groundwater resides in the porous rock body because matrix porosity is almost four orders of magnitude larger than the fracture porosity (124).
Rock samples were collected at depths of 288, 298, 310, 312, 351, and 421 m and cut into 3 inch lengths. Samples from depths of 277, 295, and 297 m were crushed. All samples were placed in vacuum-sealed bags, purged with N₂ and shipped on ice to Clemson University. Groundwater was collected at a monitoring well in the source zone and one further downgradient in the TCE plume (Appendix A.1).

3.3.2 Chemicals

The following chemicals (purity, source) were used in this study: TCE (99%, Alfa Aesar), cDCE (99%, TCI America), VC (99.5%, Fluka), polymer grade ethene (99.9%, Airgas), ethane (99.95%, Matheson), methane (99%, Matheson), hydrogen (99.995%, Airgas), and acetylene (99%, Matheson). Sodium lactate syrup was obtained from EM Science (58.8 to 61.2% sodium lactate; specific gravity 1.31). All other chemicals were reagent grade. Anaerobic mineral salts medium (MSM) was prepared as previously described (138) (Appendix A.2).

3.3.3 Intact Core Microcosms

A conceptual model for the intact core microcosms (Appendix B.1) was developed based on a similar unit used to evaluate thermal treatment of VOCs within rock (117) (Fig. 3-1). Each microcosm consists of a 3 inch (length) × 2.375 inch (diameter) rock core, with two stainless steel end caps. The cores were wrapped in Teflon tape, followed by heat shrinkable Teflon tubing. Initially, the cores were wrapped in rubberized tape confined by hose clamps, in order to force groundwater (containing 20 mg/L TCE, 1 mM bromide and 1 mg/L resazurin) through to achieve saturation.
Figure 3-1. Schematic of the intact rock core microcosms and processing of samples.
Next, the hose clamps and rubber tape were removed to reveal the Teflon sleeve. The two ends of the Teflon sleeve were shortened by 0.25 inch to expose the stainless steel so that the end caps could be welded. Each core was then inserted into a smooth-bore seamless 304 stainless steel tubing (2.5" OD, 0.065" wall thickness), with the inner wall custom machined so that the core fit as snugly as possible. The ends of the pipe were welded to the ends of the stainless steel caps using tungsten inert gas welding, forming a leak-proof seal. The welding was done at a sufficiently low temperature to avoid any disruption of the core or loss of TCE. To prevent overheating, the stainless steel pieces were partially immersed in cold water to cool down the heated area.

One of the end caps was machined to create a 0.25-inch-deep head chamber next to the end of the core. The rock core represented the matrix and the chamber mimicked a fracture. Once assembly of the cores was completed, the chamber was flushed with groundwater containing resazurin, but no TCE or bromide, to simulate a cleaned up fracture. Two Mininert® valves were then installed on the end cap (corresponding to the chamber) for sampling. A stainless plug was used to seal off the hole in the bottom cap (corresponding to the side of the rock furthest from simulated fracture end). Details regarding microcosm assembly can be found in Appendix B.1.

Twelve rock core microcosms were prepared, divided into 6 pairs. Each pair was constructed with rocks from adjacent locations in the core hole. The intent was for each pair to be as similar in characteristics, with one serving as an unamended control (U) and the other as a treatment biostimulated with lactate (L). One of the unamended controls (U4) broke during preparation, leaving only 5 unamended microcosms. The remaining
three consisted of vessel controls, i.e., they consisted for the stainless steel case and groundwater, with no rock or Teflon.

The core microcosms were incubated at room temperature (22-24 °C) in the upright position under quiescent conditions. To simulate flow through the fracture, approximately 2 mL of fresh groundwater was injected into the chamber through one of the Mininert® valves on a weekly basis, and the displaced groundwater was collected through the other valve (Appendix B.1). Groundwater added to one half of the microcosms was amended with lactate. The 2 mL samples collected were used to determine VOCs, organic acids, inorganic ions, and pH. δ^{13}C measurements were conducted at approximately three month intervals. The mass of each microcosm was also recorded before and after sampling.

At the end of the incubation period (approximately 21 months), the cores were shipped on ice to the University of Guelph where they were immediately frozen (-80 °C), removed from the stainless steel casing, and sliced into three equally sized cylinders (Fig. 3-1). Each section was then cut into quarters and used for 1) VOCs analysis at the University of Guelph; 2) molecular microbiology and organic acids analyses at Clemson University; and 3) inorganic ions at the University of Ottawa.

An additional three microcosms served as vessel controls to evaluate potential reactivity of TCE with the stainless steel. Each consisted of a stainless steel pipe and two end pieces, which were welded together and filled with groundwater containing TCE, bromide, and resazurin; lactate was not added.
3.3.4 Crushed Rock Microcosms and Electron Donor Experiments

Crushed rock microcosms were constructed with samples from the same core hole to aid in comparing the intact rock core results. Two sets of 12 crushed rock microcosms were prepared; one set received ~14 mg/L TCE (taking into account partitioning between the headspace and liquid) to mimic the average concentration within the rock core microcosms (i.e., starting TCE mass divided by the total void volume inside the microcosm, ~28 mL in the rock + 15 mL in the head chamber). The other set received ~0.7 mg/L, close to what was used in previous crushed rock microcosms (Chapter 2). Among each set, half the bottles received lactate.

Additional experiments were performed to explore the function of various electron donors. The first experiment focused on electron donors for sulfate reduction (acetate, hydrogen, and lactate). The second focused on electron donors for reductive dechlorination. An enrichment culture grown on TCE (Chapter 2) was washed to remove nutrients and substrates and used as inoculum for microcosms containing modified anaerobic MSM. Details about the experimental set up can be found in Appendix B.2.

3.3.5 Analytical Methods and CSIA

The total amount of VOCs present in the microcosms (TCE, cDCE, VC, ethene, ethane, acetylene, and methane) was monitored by gas chromatographic (GC) analysis of 0.5 mL headspace samples using a column packed with 1% SP-1000 on 60/80 Carbopack-B (140). Chloride, bromide, and sulfate were measured by an ion chromatography (IC) on an AS9-HC anion exchange column (Dionex, 4 mm x 250 mm; 9 mM Na_2CO_3 eluant, 1 mL/min). Organic acids were analyzed on a high performance
liquid chromatography (HPLC) system on an Aminex® HPX-87H ion exclusion column (300 mm x 7.8 mm; 0.01 N H$_2$SO$_4$, 0.6 mL/min) at 210 nm wavelength. Details can be found in Appendix A.4. Samples preserved with several drops of 10 M NaOH were periodically sent to the University of Waterloo Environmental Isotope Laboratory for measurement of $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE.

3.3.6 Genetic Analyses

After the sacrificing the intact rock microcosms, DNA was extracted from the groundwater in the chamber and crushed rock (~1 g) from each core section, using MOBIO PowerSoil® DNA Isolation Kit following manufacturer’s protocol. *Dehalococcoides, bvcA, vcrA* and *tceA* were quantified using the TaqMan®-Probe-based quantitative polymerase chain reaction (qPCR) method (141-142) (Appendix A.5). Other bacteria, including *Dehalobacter, Desulfitobacterium, Sulfurospirillum, Desulfuromonas, Geobacter* and *Geobacter lovleyi*, were quantified with SYBR® Green Dye-based qPCR (143-146). Detection limits and other method details are presented in Appendix A.5.

3.4. Results and Discussion

3.4.1 VOCs, Ions and Organic Acids

Lactate addition simulated reductive dechlorination of TCE to cDCE in five out of the six lactate-amended microcosms, while only one out of the five unamended microcosms dechlorinated TCE to cDCE (Table 3-1). Further reductive dechlorination of cDCE to VC and ethene was not observed.
Table 3-1. Summary of reductive dechlorination activities in the intact core microcosms.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Unamended (U)</th>
<th>Lactate Amended (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 and U1</td>
<td>TCE</td>
<td>TCE (\rightarrow) cDCE</td>
</tr>
<tr>
<td>L2 and U2</td>
<td>TCE (\rightarrow) cDCE</td>
<td>TCE (\rightarrow) cDCE</td>
</tr>
<tr>
<td>L3 and U3</td>
<td>TCE</td>
<td>TCE</td>
</tr>
<tr>
<td>L4 and U4</td>
<td>N/A (broken)</td>
<td>TCE (\rightarrow) cDCE</td>
</tr>
<tr>
<td>L5 and U5</td>
<td>TCE</td>
<td>TCE (\rightarrow) cDCE</td>
</tr>
<tr>
<td>L6 and U6</td>
<td>TCE</td>
<td>TCE (\rightarrow) cDCE</td>
</tr>
</tbody>
</table>

In the head chamber of unamended core microcosm #5 (U5), TCE and bromide concentrations both experienced a rapid increase followed by steady and slow decrease, while the sulfate concentration remained almost unchanged over time (Fig. 3-2). Similar results were observed for other unamended microcosms (Appendix B.3). Organic acids were not measured for unamended microcosms. There were low levels of TCE (0.71 mg/L) and bromide (0.04 mM) at the beginning of the incubation (Fig. 3-2, insets). This is because the first sample was taken approximately one hour after the head chamber was flushed with plain groundwater, which allowed a small amount of TCE and bromide to diffuse into the chamber. The next data point on the same day was calculated based on removal of 2 mL from the 14.5 mL chamber and replacing it with groundwater that was free of TCE and bromide; hence the vertical decrease. All of the data for VOC and inorganic ions are plotted in the same “measured-to-calculated” manner, giving rise to the “saw-tooth” pattern.
Figure 3-2. Results for (a) VOCs and (b) inorganic ions in unamended intact core microcosm U5. The color bar below the legend in (a) indicates the color of the resazurin in weekly groundwater samples.
After one week of incubation, the concentration of TCE and bromide increased to 2.16 mg/L and 0.26 mM, respectively (Fig. 3-2, insets). These rapid increases were inferred to be the result of the significant concentration gradients between the groundwater in the chamber and the pore water inside the rock matrix. The concentrations in the chamber soon started decreasing, probably because the concentration gradient and diffusion rates lessened, while the volumetric rate of exchange for groundwater in the chamber remained constant.

There was no indication of reductive dechlorination of TCE in U5 during the 594 days of monitoring. The trend for bromide tracer was similar to TCE (Fig. 3-2b), confirming that changes in TCE concentrations were primarily caused by diffusion rather than degradation. The lack of reductive dechlorination is consistent with the observation that the redox potential remained above -110 mV (as indicated by resazurin being pink most of the time) and methane remained below 0.07 mg/L. Sulfate remained at relatively constant levels, suggesting that the concentration in the rock matrix and the fracture were similar and therefore no diffusion occurred. It also suggests an absence of sulfate reduction, which is consistent with the lack of electron donor.

Data for paired lactate-amended core microcosm (L5) are presented in the same manner as for U5, i.e., there are two data points for each day (Fig. 3-3). Lactate is an exception, as the measured value is lower than the calculated value that follows it. This occurred because lactate was always present at a higher concentration in the groundwater being added compared to the sample being displaced. TCE started at 0.47 mg/L and rapidly increased to a peak of 2.70 mg/L, followed by a slow decrease. Results for the
Figure 3-3. Results for (a) VOCs, (b) inorganic ions, and (c) organic acids in lactate-amended intact core microcosm L5. The color bar below the legend in panel (a) indicates the color of the resazurin in weekly groundwater samples.
first 126 days were similar to U5. Thereafter, reductive dechlorination of TCE to cDCE began, such that by day 200, TCE was approximately 0.01 mg/L. cDCE increased and peaked at 3.34 mg/L around day 223, followed by a gradual decline (Fig. 3-3a). The onset of reductive dechlorination in this core was coincident with a change in the color of the groundwater samples from pink to clear around day 50, indicating that the redox potential was reduced to below -100 mV. VC and ethene were not detected.

Bromide in L5 behaved similarly to U5, indicative of similar rates of diffusion from the paired rock samples. Starting at 0.07 mM, bromide increased to 0.30 mM after two weeks and gradually decreased. Unlike U5, sulfate consumption started in L5 soon after the core was set up and rapidly dropped to below detection; weekly increases were attributable to the addition of site groundwater with ~1.5 mM sulfate, which was repeatedly consumed, concurrent with reductive dechlorination of TCE to cDCE.

Lactate consumption started immediately and the concentrations in the head chamber decreased until lactate was close to or below detection around day 40, and further additions were repeatedly consumed within one week (Fig. 3-3c). Acetate accumulation started on day 20. Lactate was assumed to be used up by other processes such as sulfate reduction, or acetate was produced but consumed during the first few weeks. After day 20, acetate started to build up but soon leveled off at below 0.7 mM. In an attempt to stimulate cDCE reduction to VC, the concentration of lactate in the groundwater was increased from 2 to 4 mM on day 196. This led to a small residual lactate level after each week, but with on-going acetate accumulation. The lactate dose was increased to 5 mM on day 307. Acetate continued to accumulate and peaked on day
524 at 4.6 mM. Propionate was detected starting on day 377. Prior HPLC analysis cannot be used to quantify propionate due to problems with the HPLC column (i.e., a “ghost peak” eluted at the same time as propionate). Propionate mostly increased during the period of highest lactate addition, but its maximum level (0.34 mM) was more than an order of magnitude lower than the peak for acetate. Accumulation of propionate is an indicator of excess hydrogen from the fermentation of lactate (160). This suggests that the lack of reductive dechlorination of cDCE to VC or ethene was not a consequence of an inadequate availability of electron donor.

Trends in the other microcosms were similar, with notable exceptions (Table 3-1). Reductive dechlorination of TCE to cDCE occurred in one of the five unamended microcosms, consistent with the occasional dechlorination activity in unamended crushed rock microcosms (Chapter 2). TCE to cDCE was observed in five of the six lactate amended microcosms. Unlike the previously prepared crushed rock microcosms (Chapter 2), there was no further reduction to VC or ethene.

Methane was absent from any of the unamended microcosms (Appendix B.6), consistent with the presumption that these microcosms were deficient in electron donor. Methane accumulation occurred in L1, L3, and L4, but only after at least 300 days of incubation when the lactate dose was increased. In two of the microcosms with active reductive dechlorination (L1 and L4), methane began to accumulate when TCE was close to the detection level in the head chamber.

Acetylene accumulated but at levels too low to be visible in Figure 3-2 and 3-3. Data are shown in Appendix B.6. Acetylene remained below 0.02 mg/L in the
unamended microcosms. Levels were consistently higher in the lactate-amended microcosms. The lower redox potential may have created an environment more favorable for abiotic degradation. Notably, acetylene levels were highest in the microcosms with limited (L1) or no (L3) reductive dechlorination. Acetylene levels started to increase in these microcosms around the same time that the methane concentration increased.

Acetate accumulation started soon after sulfate levels decreased; sulfate was kept low by repeated additions of lactate. The chemical oxygen demand (COD) associated with acetate production and sulfate consumption was less than half of the lactate COD consumed. Methane and propionate production and reductive dechlorination of TCE contributed only a minor amount to lactate consumption, suggesting that a significant fraction of the COD diffused into the rock matrix.

3.4.2 Enrichment of $\delta^{13}C$

Results for $\delta^{13}C$-TCE are shown in Figure 3-4a for microcosms with no appreciable reduction of TCE to cDCE (U3, U5, U6, and L3). U1 was not tested because its TCE concentration was too low. Also shown are the results for 1) triplicate water controls (WC), consisting of the same groundwater that was used on a weekly basis for exchange of water in the head chamber of the microcosms, plus freshly added TCE saturated groundwater prior to each analysis; and 2) triplicate container controls (SS), consisting of groundwater incubated within stainless steel cylinders with the same dimensions as the microcosms. Average $\delta^{13}C$-TCE results for all of the water controls was used in place of a time zero measurement for $^{13}C$-TCE (Fig. 3-4a). There was no statistically significant change in the WC values or the SS container controls ($p>>0.05$
Figure 3-4. $\delta^{13}$C enrichment results for intact core microcosms based on (a) $\delta^{13}$C-TCE levels in individual bottles without or prior to the onset of reductive dechlorination and (b) $\delta^{13}$C-cDCE levels in individual bottles with cDCE production. WC stands for water controls. SS stands for stainless steel container controls, which were only tested twice at the end.
for the trend lines), while a significant increase in δ\textsuperscript{13}C-TCE (up to 5‰) occurred in the four microcosms, at rates ranging from 0.002 to 0.006 ‰/day.

Results for δ\textsuperscript{13}C-cDCE are shown in Figure 3-4b for microcosms that underwent reductive dechlorination of TCE to cDCE, including U2, L2, L4, L5, and L6. L1 was not tested because its cDCE concentration was too low. Enrichment in δ\textsuperscript{13}C-cDCE occurred in all of the microcosms at rates ranging from 0.002 to 0.011 ‰/day, consistent with the rates in previously evaluated crushed rock microcosms (Chapter 2).

In a few of the microcosms, the increases in δ\textsuperscript{13}C-TCE or δ\textsuperscript{13}C-cDCE over the early incubation period were followed by a plateau in some of the cores (Fig. 3-4). This may have been caused by a depletion of reducing capacity of iron-bearing minerals, as observed in a previous crushed rock microcosm study (137), which may explain the plateau in δ\textsuperscript{13}C in certain microcosms. Nevertheless, the overall trend towards enrichment was statistically significant in all of the cores shown.

Since \textsuperscript{14}C-labeled compounds were not added to the intact core microcosms, the only evidence in support of abiotic transformation was enrichment in δ\textsuperscript{13}C-TCE and δ\textsuperscript{13}C-cDCE. Results for crushed rock microcosms (Chapter 2) suggested that accumulation of \textsuperscript{14}CO\textsubscript{2} and \textsuperscript{14}C-NSR correlated with enrichment in δ\textsuperscript{13}C-TCE and δ\textsuperscript{13}C-cDCE. By extension, enrichment in δ\textsuperscript{13}C-TCE and δ\textsuperscript{13}C-cDCE in the intact core microcosms was likely a result of abiotic transformation processes that produced CO\textsubscript{2} and NSR as products.
3.4.3 pH and Mass Change

Average pH ranged from 7.73 to 7.90 in unamended intact core microcosms and from 7.51 to 7.73 in lactate-amended microcosms. Overall, the pH in the lactate-amended microcosms was slightly lower than in the unamended ones, likely due to the greater level of carbon dioxide production associated with metabolism of lactate and acetate, as well as the use of lactic acid together with sodium lactate. Nevertheless, the pH levels in all microcosms remained in a range that is conducive to biotic reductive dechlorination (Appendix B.8).

The total mass of the microcosms was recorded before and after making the weekly exchange of groundwater, to ensure that the sample collected was properly displaced by the groundwater injected, and to reveal if a leak developed. There was an increase over the full incubation period, ranging from of 1.3 to 5.4 g per core (Appendix B.8). The mass increase primarily occurred during several of the sampling events, i.e., when less than 2 mL of groundwater was pushed out from the chamber after 2 mL was injected. This was likely a consequence of displacing gas bubbles in the head chamber. The mass increases were compared to the mass of water that should have been present if the cores were uniformly saturated (28 g), indicating that an average of 89.2±3.5% (α =0.05) of the cores were saturated (Appendix B.8).

3.4.4 End of Incubation Evaluation

After the microcosms were sacrificed, part of the core samples were crushed, dried and rehydrated to extract non-volatile compounds. The mass of wet rock and dry rock were measured, which allowed calculation of a water-to-rock mass ratio specific to
each rock section (Fig. 3-5a). Comparison of the three core sections from each microcosm suggested uniform water distribution from top to the bottom. The cores in each pair exhibited similar water-to-rock mass ratios, indicating that the porosities and saturation levels were similar between the pairs. Using a dry rock bulk density for this site of 2290 kg/m$^3$, the porosity was estimated at 8.2% to 16.2%, with an average of 13.2%. This is close to the value for sandstone (13%) reported at the site (161).

Another important observation was that microcosm pair #1 had significantly lower water-to-rock mass ratios compared to the other pairs, which was indicative of lower porosities in rock obtained from this depth.

Bromide concentrations decreased from the rock bottom to the head chamber (Fig. 3-5b), consistent with the presumed direction of diffusion. Acetate was the major organic acid identified in the sacrificed cores (Fig. 3-5c); lactate and formate were also present but at much lower concentrations (Appendix B.9). As expected, acetate decreased from the head chamber to the bottom of the core in lactate-amended microcosms, because acetate was generated from lactate added to the head chamber, which subsequently diffused into the core.

Geobacter and total bacteria were quantified in the sacrificed cores through qPCR analysis (Appendix B.9), while Dehalococcoides, vcrA, bvcA, tceA, and Geobacter lovleyi were below detection. When comparing the gene copy concentrations in the head chambers, Geobacter and total bacteria were higher in the lactated-amended microcosms than in unamended microcosms ($p<0.05$, one-tail Student’s $t$-test), but no difference was found for the core sections. Results for the core sections exhibited more variations,
Figure 3-5. Results for end-of-incubation analyses of (a) water to rock mass ratio, (b) bromide distribution and (c) acetate distribution. The three or four bars for each microcosm represent (from left to right) the concentrations in the reservoir inside the head chamber (C) and inside the top (T), middle (M) and bottom (B) rock sections. Water-to-rock mass ratio is not relevant for the chamber.
possibly due to the heterogeneity of microbial distribution, the sample size being too small (~1 g) to fully represent the entire core section, and decay of microbes by the time the cores were measured.

3.4.5 Mass Balances for Bromide, TCE and cDCE

A mass balance for bromide was determined based on cumulative removal during sampling and the amount recovered in the final analysis, assuming a starting amount of 28.3 μmol/microcosm (Fig. 3-6a). Recoveries ranged from 66% to 102% for pairs #2-6, and 37% to 47% for pair #1. An overestimation of porosity likely caused the lower recovery in pair #1, as suggested by their lower water-to-rock mass ratio (Fig. 3-5a).

Cumulative removal of TCE and cDCE (expressed in μmoles per microcosm) as a consequence of weekly sampling are shown in Figure 3-6b. For pairs #1, #5 and #6, the onset of reductive dechlorination in lactate-amended microcosms led to higher removal ratios of the chlorinated ethenes than the corresponding unamended microcosms. This is likely because cDCE diffuses at a higher rate and adsorbs to a lesser extent than TCE, allowing more mass to enter the head chamber and be removed. For pair #2, reductive dechlorination occurred in both, which coincided with a high percent recovery of TCE plus cDCE. For pair #3, the absence of reductive dechlorination in both microcosms resulted in a lower recovery for the mass balance.

3.4.6 Core Saturation

Several lines of evidence suggest the cores were close to full saturation following preparation of the microcosms: 1) The mass ratio of water-to-rock indicated a uniform
Figure 3-6. (a) Recovery of bromide and (b) removal of total chlorinated ethenes at the end of the incubation period and the corresponding percent recovery/removal from each microcosm.
distribution of water and the calculated porosity was close to what has been reported for the site; 2) The mass balance for bromide in 9 of the 11 microcosms yielded an 84±8% (α=0.05) recovery, assuming uniform saturation with 1 mM bromide and 13% porosity. Bromide recovery in the remaining two microcosms (U1 and L1) was lower (37-47%), likely due to an overestimation of their porosity and consistent with their lower water-to-rock mass ratio; 3) The mass balance for total chlorinated ethenes in the five cores that exhibited TCE reduction to cDCE was 92±6% (α=0.05), assuming a uniform saturation with 20 mg/L of TCE and 13% porosity. Recoveries were lower in microcosms without reductive dechlorination, suggesting that a significant amount of the TCE still resided within the cores. Since VOC data for sacrificed microcosms are not yet available, a full mass balance was not evaluated; and 4) The mass gain in the microcosms during operation allowed for an estimate of an average water saturation level of 89 ± 4% (α=0.05). The percentage of void pore volume (~11%) is similar to the deficit in the bromide mass balance (~16%). Taken together, these metrics suggest that groundwater forced through the microcosms during preparation reached 84 to 89% of the pore spaces.

3.4.7 Crushed Rock Microcosms

Crushed rock microcosms were prepared at the same time as the intact rock core microcosms, using rock from adjoining parts of the same borehole. TCE underwent a relatively slow rate of reductive dechlorination to cDCE in 3 out of the 12 unamended microcosms, and much faster reduction to cDCE in 9 out of the 12 lactate-amended microcosms. The onset of reductive dechlorination was faster in the bottles that were started with a lower initial concentration of TCE, suggesting that the higher concentration
added to the intact core microcosms may have been moderately inhibitory. Only one bottle with 0.7 mg/L TCE exhibited complete reduction to ethene. These results were consistent with the lack of cDCE reductive dechlorination in the intact rock core microcosms and the lack of *Dehalococcoides* in the rock samples (Appendix B.10).

### 3.4.8 Lactate, Acetate and Hydrogen as Electron Donors

Lactate, acetate, and hydrogen were evaluated as electron donors for sulfate reduction and reductive dechlorination. Acetate was of particular interest, since it was a major product from biodegradation of lactate, accumulated to an appreciable level in the chamber, and diffused deeply into the cores. Using inoculum from the crushed rock microcosms described above, lactate and hydrogen supported sulfate reduction, while only one of the six acetate-amended microcosms exhibited sulfate consumption (Appendix B.11).

Electron donors for reductive dechlorination were evaluated with the TCE enrichment culture described in Chapter 2. TCE reduction to cDCE was equally supported by lactate, acetate and hydrogen. This is consistent with the evidence suggesting that *Geobacter* are the major dechlorinators of TCE to cDCE at this site; they are known to use a variety of electron donors. Production of VC but not ethene was also observed, with lactate being the most effective. The fact that acetate supported dechlorination of cDCE was unexpected, since the *Dehalococcoides* that mediate this reaction are only known to use hydrogen as their electron donor. It is presumed that acetate supported reductive dechlorination of cDCE to VC via its oxidation to CO₂ and hydrogen, because there was some evidence in support of hydrogen accumulation in the
acetate amended treatment; presumably the acetate oxidizers were present at too low a level to support sulfate reduction via hydrogen production (Appendix B.11).

A summary of potential pathways is shown in Figure 3-7. Lactate and hydrogen supported sulfate reduction, while acetate did not. Hydrogen, lactate, and acetate supported reductive dechlorination of TCE to cDCE and likely VC. This indicates that acetate production in the intact core microcosms and its diffusion into the rock matrix may have supported TCE removal. It is not clear if the propionate and formate formed from lactate yielded hydrogen that supported reductive dechlorination and sulfate reduction.

Figure 3-7. Summary based on pathway experiments. A check mark indicates that the reaction was confirmed, a question mark indicates that the occurrence of this reaction is still in doubt, and a cross indicates the reaction did not occur in either pathway experiment. HCOOH = formic acid; CH3COOH = acetic acid; CH3CHOHCOOH = lactic acid; CH3CH2COOH = propionic acid.
3.5. **Conclusions**

Delivery of lactate to the head chamber, which mimics flow through a fracture, in an intact core microcosm created a reduced environment and led to complete sulfate reduction. None of the five unamended microcosms had sulfate reduction and the redox potential was consistently higher than -100 mV. A high rate of TCE reduction to cDCE occurred in five out of the six lactate-amended microcosms and one out of unamended microcosms. These results were generally consistent with previous crushed rock microcosms (Chapter 2) as well as the ones evaluated in this study.

Even with lactate addition, there was no apparent reduction of cDCE to VC and ethene in the intact core microcosms, unlike previous results with crushed rock microcosms in which lactate addition stimulated several microcosms to generate VC and ethene (Chapter 2). However, these are not contradictory observations, as both can be explained by heterogeneous distribution of halorespiring microbes, especially *Dehalococcoides*, in the sandstone at this site. Given the limited number of rock cores tested in this study, it is reasonable to assume that they happen to locate in zones where indigenous *Dehalococcoides* are absent, and thus may not be representative of other locations at the site. The corresponding crushed rock microcosms confirmed this, as only one out of the 12 lactate-amended bottles exhibited ethene formation, and to a lesser extent than what was observed in the prior crushed rock microcosms (Chapter 2). Another possibility is that the initial concentration of TCE infused into the rock (~20 mg/L) was inhibitory to *Dehalococcoides*; this may also explain why the only incidence
of VC and ethene production in the corresponding crushed rock microcosms occurred in a bottle with a low initial level of TCE (~0.7 mg/L).

Though further biodegradation beyond cDCE did not occur, reductive dechlorination appears to enhance total chlorinated ethene removal from the rock by reducing TCE levels in the fracture and speeding up the back diffusion process, especially for cDCE. In this regard, biostimulation could contribute to the release of TCE from the matrix to the fracture, and thereby shorten the overall remediation time.

Enrichment in $\delta^{13}C\text{-TCE}$ occurred in microcosms that did not undergo a significant level of reductive dechlorination; enrichment in $\delta^{13}C\text{-cDCE}$ also occurred with cDCE formed via reductive dechlorination of TCE, suggesting that the abiotic transformation processes observed in previous crushed rock microcosm study (Chapter 2) also occurred in the intact cores. Because $^{14}C\text{-TCE}$ was not used, it was not possible to estimate a rate of degradation based on accumulation of $^{14}CO_2$ plus $^{14}C\text{-NSR}$, as in the previous crushed rock microcosms. Furthermore, the VOC and $\delta^{13}C$ results cannot be used directly for rate estimation because they represent the combined effect of multiple processes, including diffusion, absorption, and reaction. Prediction of rates from the intact rock core microcosms will require numerical simulation of the multiple processes involved that may contribute to enrichment in $\delta^{13}C$.

The novel intact core microcosm developed for this study met the objective of evaluating natural attenuation and biostimulation in a simulated fractured-rock environment. It successfully addressed several key design issues associated with this novel type of microcosm. First, the flexible Teflon tape and heat-shrinkable Teflon sleeve
that sealed the uneven rock surface effectively prevented annular flow. Teflon is preferable to the epoxy glue used in other studies (113-116) since it is non-toxic and inert. Second, the method used to confine the core during pumping (i.e., silicone tape and hose clamps) facilitated the process of saturating the cores with a nearly uniform contaminant distribution. Third, the welded stainless steel case created a leak-proof system that limited diffusive loss of VOCs. Lastly, the method used to simulate flow of clean water over a fracture permitted monitoring of the groundwater impacted by back diffusion and the efficacy of biostimulation for TCE remediation in the rock matrix.
4. REMEDIATION OF CHLORINATED ETHENES IN FRACTURED SANDSTONE WITH NATURAL ATTENUATION AND BIOSTIMULATION: MODEL DEVELOPMENT AND NUMERICAL SOLUTION

4.1. Abstract

Two microcosm studies were conducted for a TCE-contaminated industrial site underlain by a fractured sandstone aquifer to evaluate natural attenuation and biostimulation as potential remediation stratagems of chlorinated ethenes. One study used crushed rock and the other employed intact core. Both studies documented the occurrence of biotic and abiotic pathways and demonstrated the potential of using lactate to enhance reductive dechlorination. Using intact core allowed better simulation of in situ conditions and diffusion processes, and therefore may provide valuable information for site remediation, such as diffusion coefficients and degradation rates. However, data collection and interpretation are challenging due to the inherent heterogeneity of intact core microcosms. Modern modeling techniques have become increasingly useful in studying contaminant transport and fate in heterogeneous media, and by extension, facilitate prediction of plume behavior and remediation timelines. The objective of this study was to numerically simulate reaction and transport of contaminants in the intact core microcosms and obtain site-specific parameters.

A model was developed in a 2D radial symmetrical system using COMSOL Multiphysics and it was capable of simulating diffusion, biotic/abiotic reaction and isotope fractionation processes within a dual porosity (core-fracture) system. The model
was calibrated with data for TCE and δ\textsuperscript{13}C-TCE, cDCE and δ\textsuperscript{13}C-cDCE, and bromide. It was then used to estimate several parameters, including rock diffusivity, Monod equation constants, and abiotic transformation rates. This greatly enhanced the relevancy and applicability of the intact core microcosms to the actual site. In the future this core-scale model could be used together or integrated into the existing site-scale model for better data analysis and trend prediction. However, it should be noted that model-predicated values came with a level of uncertainty, e.g., the estimates of abiotic transformation rates were very sensitive to the underlying assumptions about enrichment factors, and therefore should be used with caution. Such uncertainties may be removed through further field analysis and experimentation.

4.2. Introduction

Fractured bedrock aquifers pose challenges to remediation due to the presence of low permeable zones and complicated flow systems (1-4, 16, 112, 130-131, 155). Mass transport is strongly affected by matrix diffusion, compared to the advection-dispersion controlled flow in unconsolidated aquifers. Therefore aggressive treatment technologies, such as pump and treat, may not work effectively because they primarily target contaminants in the fracture where flow is active, while the majority of mass often resides in the rock matrix. Monitored natural attenuation and biostimulation are considered more cost-effective in situ remediation stratagems for fractured bedrock aquifer and have become the focus for many field and laboratory studies (137). Challenges are presented in documentation of individual pathways when multiple processes, including diffusion, bio/abiotic reactions, adsorption, and volatilization exist.
together and potentially influence each other. Traditional treatability studies often use microcosms containing groundwater and crushed rock to replicate the in situ condition, but it is unclear how crushing might affect the rate and transformation capacity, when microbiology, mineralogy and geochemistry are potentially altered. Experiments using intact pieces of rock would be more representative, but few studies have been conducted due to the difficulty of system set up and monitoring (115-118). Besides, data interpretation may not be as straightforward as for homogenous crushed rock microcosms due to the coupling and integration of different processes. Advances in modeling methods have facilitated the study of multiple processes in heterogeneous environments.

Three conceptualizations have been used to describe the flow system within fractured bedrock aquifers by treating them as an equivalent porous continuum, a discrete fracture network, or dual porosity media (119). Simulations of groundwater flow and solute transport/reaction are generally achieved with numerical models or analytical models, or a hybrid of the two. Compared to analytical models, numerical models are mathematically simpler, more versatile, and better suited for computation (162-163). Well known computer codes for groundwater modeling include MODFLOW, HydroGeoSphere and FEFLOW (120, 164-165). One potential drawback of numerical modeling is that accurate simulation requires extremely high spatial and temporal discretization to capture the system geometry and concentration gradients (16). Conversely, analytical models simulate contaminant transport assuming a simple uniform groundwater flow field and are therefore easier to implement, but also make it less appropriate for complex systems like fracture bedrock aquifers (162-163). Examples of
analytical plume models are REMChlor, BIOCHLOR and BIOSCREEN (121, 166-168). Several semi-analytical approaches were developed to enable simulation of more complicated and heterogeneous environments (122, 169-170).

A fracture sandstone aquifer in southern California is contaminated with TCE. Multiple field and laboratory studies have demonstrated that a combination of abiotic and biotic transformation processes are responsible for attenuation of TCE (63, 128, 136). A generic conceptual model was described by Parker et al. (130-131), which includes dissolution of TCE DNAPL in fractures and subsequent diffusion of dissolved TCE into the rock matrix. Then a combination of a 2D discrete fracture network model (using FRACTRAN or HydroGeoSphere) and a 3D equivalent porous media model (using MODFLOW) was employed to simulate steady-state groundwater flow and transient solute transport with site-derived parameters (17-18). The simulations show strong plume retardation due to matrix diffusion during the early stage following contamination (<50 years) and a dramatic impact on plume behavior by different biotic and/or abiotic degradation rates later on (>50 years). The results suggest that a slow rate of degradation in the rock matrix (equivalent to a half-life of 20 yr) may be sufficient for plume attenuation over a long period. The degradation rate constant is critical for the prediction of the influence of natural attenuation; however, there has not been adequate data from this site to make reliable rate estimation. Transformation rates of 0.038 yr⁻¹ for TCE and 0.044 yr⁻¹ for cDCE were observed in crushed rock microcosms (Chapter 2), but the accuracy may be compromised by the crushing processes. A novel microcosm study was conducted using intact cores from the site that better represent in situ conditions (Chapter
3). Natural attenuation and biostimulation were observed. The purpose of this study was to develop a numerical model that simulates diffusion, transformation reactions and isotope fractionation in a dual porosity domain, which were then used to estimate site-specific parameters such as rock diffusivity, Monod equation constants, and biotic and abiotic transformation rates. COMSOL Multiphysics® was used to perform this task.

4.3. **Intact Core Microcosms Study**

Design and operation of the intact rock core microcosms is described in Chapter 3. Briefly, each microcosm consisted of a sandstone core (3” long, 2.375” diameter) sandwiched between two stainless steel end caps of same diameter. One of the caps was hollowed out to create a head chamber to simulate a fracture with groundwater flow. Core and end caps were encased in a heat-shrinkable Teflon sleeve. Site groundwater amended with approximately 20 mg/L of TCE, 1 mM bromide (conservative tracer) and resazurin (redox indicator) was forced through the rock under pressure to create the initial conditions. The core and end caps were slid into a stainless steel pipe with minimum gap around the core and the ends of the pipe were welded to the caps. The end caps with the hollowed out portion were then flushed with groundwater containing no TCE or bromide, and fitted with two Mininert® sampling valves. Once per week, 2 mL of groundwater was injected through one valve and the displaced groundwater was collected from the other valve in a serum bottle, simulating clean fracture flow over the contaminated rock matrix. Six of the microcosms received groundwater amended with lactate; the other set received plain groundwater. Samples were analyzed weekly for VOCs, bromide, sulfate, organic acids, and pH. δ¹³C was measured every 3-4 months to assess abiotic reaction.
Reductive dechlorination of TCE to cDCE occurred in five of the lactate-amended microcosms and one of the unamended microcosms. However, no reduction to VC or ethene occurred as in previous crushed rock studies. The presence of other transformation processes (potentially abiotic) was confirmed based on up to 5‰ enrichment of $\delta^{13}$C-TCE in microcosms where TCE didn’t undergo reductive dechlorination, and up to 4‰ enrichment of $\delta^{13}$C-cDCE in ones where TCE was reduced to cDCE. The cores were operated for over 20 months before being disassembled for final analysis.

4.4. Model Development

4.4.1 Geometry, Boundary Conditions and Simplifications

To simplify the model set up, the 3D cylindrical geometry of the intact rock core microcosm was approximated using a 2D radial symmetric formulation, also called a 2D r-z system because the coordinates are radius (r) and height (z) (Fig. 4-1). The model domain was divided into a chamber section (top) and a rock section (bottom). The geometries were set up according to the actual dimensions of the microcosms: head chamber radius = 2.699 cm (1.063 inch), height = 0.635 cm (0.25 inch); core radius = 3.016 cm (2.375 inch), height = 7.620 cm (3.0 inch).

A simplification was applied to simulate the sampling process. The microcosms were incubated without flow except for the once per week sampling that lasted about 20 to 30 s, when 2 mL of water in the head chamber was displaced by “clean” groundwater injected through one of the Mininert® valves. An initial simulation was set up in COMSOL to follow this weekly exchange of water. While it was possible to simulate the
Figure 4-1. Representation of the intact rock core microcosms; (a) 3D geometry of the core; (b) translation of the 3D geometry to 2D r-z coordinates; (c) hypothetical simulation results for the 2D model, with colors representing different concentrations of TCE; and (d) translation of the 2D results back to the 3D geometry.
incubation period between flow events, there was no automatic way to simulate the weekly exchange of water. Consequently, the effect of groundwater exchange on the concentration in the head chamber was calculated manually for each sampling event and the result was used as the initial condition to simulate what occurred over the next week of static incubation. This approach is referred to hereafter as the “weekly model.”

The “weekly model” approach is tedious because of the manual calculation step, and a change to any single sampling event required manually recalculating the changes in concentration for all subsequent weeks. Therefore, an alternative modeling approach was developed by assuming a continuous pseudo first-order decay rate that achieved the same extent of removal of analytes that the physical exchange of groundwater achieved on a weekly basis:

$$\frac{dC}{dt} = -k_{chamber} C$$ (4.1)

where $C$ is the concentration for solute (Br$, TCE$ or cDCE), $t$ is time, and $k_{chamber}$ is a first-order rate coefficient for solute removal from the chamber. For removal of bacteria as a consequence of sampling, $C$ is replaced by $X$ (concentration of bacteria). Hereafter, this is referred to as the “continuous model.” Comparisons showed that the weekly model and continuous model behaved similarly (Appendix C.1).

Modeling of different reactive and transport processes is described in more detail in the following sections. For purpose of preliminary modeling, parameter values were assigned to those processes, based on the literature, information from the site, modeling work, and experience, as summarized in Table 4-1.
Table 4-1. Parameters used in preliminary modeling of the intact rock core microcosms.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>units</th>
<th>Parameter Description (source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T )</td>
<td>5.5E+07</td>
<td>s</td>
<td>Model simulation time-frame</td>
</tr>
<tr>
<td>( \theta )</td>
<td>13%</td>
<td>dimensionless</td>
<td>Site sandstone matrix porosity (161)</td>
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<tr>
<td>( K_{\text{chamber}} )</td>
<td>1E-08</td>
<td>m/s</td>
<td>Hydraulic conductivity in chamber</td>
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<td>( k_{\text{sampling, Br}} )</td>
<td>2.3E-07</td>
<td>s(^{-1})</td>
<td>Sampling removal rate for bromide</td>
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<td>( k_{\text{sampling, other}} )</td>
<td>1.7E-07</td>
<td>s(^{-1})</td>
<td>Sampling removal rate for VOC and microbes</td>
</tr>
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<td>( C_{\text{Br,0, chamber}} )</td>
<td>1 mM</td>
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<td>Initial bromide level in the fracture</td>
</tr>
<tr>
<td>( C_{\text{Br,0, core}} )</td>
<td>0 mM</td>
<td></td>
<td>Initial bromide concentration in the pore space</td>
</tr>
<tr>
<td>( C_{\text{TCE,0, chamber}} )</td>
<td>20 mg/L</td>
<td></td>
<td>Initial TCE level in the fracture</td>
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<tr>
<td>( C_{\text{TCE,0, core}} )</td>
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<td></td>
<td>Initial TCE concentration in the pore space</td>
</tr>
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<td>( \delta^{13}_{\text{TCE,o}} )</td>
<td>-28.4‰</td>
<td>dimensionless</td>
<td>Initial ( ^{13}\text{C} ) value for TCE</td>
</tr>
<tr>
<td>( \kappa_{\text{Br}} )</td>
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<td>m(^2)/s</td>
<td>Diffusivity of bromide in rock (171)</td>
</tr>
<tr>
<td>( \kappa'_{\text{TCE}} )</td>
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<td>m(^2)/s</td>
<td>Diffusivity of ( ^{12}\text{C}-\text{TCE} ) in rock (172-173)</td>
</tr>
<tr>
<td>( \kappa'_{\text{DCE}} )</td>
<td>1.5E-11</td>
<td>m(^2)/s</td>
<td>Diffusivity of ( ^{12}\text{C})-cDCE in rock (172-173)</td>
</tr>
<tr>
<td>( \lambda_{\text{e,microbe}} )</td>
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<td>m(^3)/s</td>
<td>Effective motility coefficient of microbes in rock</td>
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<td>m(^2)/s</td>
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<td>Enrichment factor for TCE diffusion (174)</td>
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<td>( \varepsilon_{\text{DCE,diff}} )</td>
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<td>dimensionless</td>
<td>Enrichment factor for cDCE diffusion (174)</td>
</tr>
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<td>dimensionless</td>
<td>Stalling factor during electron donor deficit</td>
</tr>
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<td>( t_{\text{min}} )</td>
<td>N/A</td>
<td>s</td>
<td>Stalling period starting time</td>
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<tr>
<td>( t_{\text{max}} )</td>
<td>N/A</td>
<td>s</td>
<td>Stalling period end time</td>
</tr>
<tr>
<td>( Y_{\text{TCE}} )</td>
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<td></td>
<td>Yield for TCE (139, 175-180)</td>
</tr>
<tr>
<td>( \mu'_{\text{max}} )</td>
<td>9.0E-07</td>
<td>s(^{-1})</td>
<td>Maximum specific growth rate for ( ^{12}\text{C}-\text{TCE} ) (175-178, 180)</td>
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<td>dimensionless</td>
<td>Enrichment factor for TCE biodegradation (71, 79, 181)</td>
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<td>( K_S )</td>
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<td>Half saturation coefficient (175-178, 180)</td>
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<td>( d_H )</td>
<td>4E-07 s(^{-1})</td>
<td></td>
<td>Endogenous decay rate (175, 177)</td>
</tr>
<tr>
<td>( X_{\text{core}} )</td>
<td>1.5E+06 copies/L</td>
<td></td>
<td>Initial biomass in the core</td>
</tr>
<tr>
<td>( X_{\text{chamber}} )</td>
<td>2E+07 copies/L</td>
<td></td>
<td>Initial biomass in the chamber</td>
</tr>
<tr>
<td>( T_{\text{lag}} )</td>
<td>3.02E+06 s</td>
<td></td>
<td>Lag time for biodegradation</td>
</tr>
<tr>
<td>( k'_{\text{TCE}} )</td>
<td>1.6E-09</td>
<td>s(^{-1})</td>
<td>1(^{st}) order abiotic reaction rate constant for ( ^{12}\text{C}-\text{TCE} )</td>
</tr>
<tr>
<td>( t_{1/2,TCE} )</td>
<td>13.7 yr</td>
<td></td>
<td>Half-life for ( ^{12}\text{C}-\text{TCE} )</td>
</tr>
<tr>
<td>( k'_{\text{DCE}} )</td>
<td>1.4E-09</td>
<td>s(^{-1})</td>
<td>1(^{st}) order abiotic reaction rate constant for ( ^{12}\text{C})-cDCE</td>
</tr>
<tr>
<td>( t_{1/2,DCE} )</td>
<td>15.7 yr</td>
<td></td>
<td>Half-life for ( ^{12}\text{C})-cDCE</td>
</tr>
<tr>
<td>( \varepsilon_{\text{TCE,abio}} )</td>
<td>-25‰</td>
<td>dimensionless</td>
<td>Enrichment factor for TCE abiotic transformation (79, 100)</td>
</tr>
<tr>
<td>( \varepsilon_{\text{DCE,abio}} )</td>
<td>-25‰</td>
<td>dimensionless</td>
<td>Enrichment factor for cDCE abiotic transformation (79, 100)</td>
</tr>
<tr>
<td>( R )</td>
<td>1.2</td>
<td>dimensionless</td>
<td>Reaction retardation factor due to matrix adsorption</td>
</tr>
</tbody>
</table>
4.4.2 Governing Equations: Solute Diffusion and Microbial Movement

A mass balance for diffusion in the intact rock core can be described using Fick’s second law of diffusion:

\[
\frac{\partial C}{\partial t} = \frac{\tau D_F}{R} \left[ \frac{\partial^2 C}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \right]
\]

(4.2)

where \( \tau \) is the matrix tortuosity, \( D_F \) is the diffusion coefficient for solute in free water, \( R \) is the matrix retardation factor, \( z \) is the distance along the length of the core, and \( r \) is the distance from the centerline. The value of \( \tau \) depends on characteristics of the porous medium and ranges between 0 and 1. Sometimes the inverse of \( \tau \) is used to represent tortuosity, which in turn acts as the denominator in equation 4.2 instead of the numerator. The retardation factor depends on characteristics of the porous medium and interactions of the solutes with the inner surfaces, with values equal to or larger than 1. Diffusion coefficients for a certain solute at room temperature are available in the literature, e.g., 1.9E-9 m²/s for bromide (182), 0.91E-9 m²/s for TCE (183), and 1.13E-9 m²/s for cDCE (183); somewhat different values are found in different sources. Tortuosity and/or retardation factors for solutes in the rock samples at this site were previously determined (directly or indirectly) for chloride, MnO₄⁻, tritiated water (HTO) and TCE (171-173, 184), but no values were available for cDCE and bromide. For the purposes of this study, values of \( \tau \), \( D_F \) and \( R \) were not evaluated separately; instead, they were lumped together into a single parameter called diffusivity (\( \kappa \)) (122):

\[
\kappa = \frac{\tau D_F}{R}
\]

(4.3)
Substituting equation 4.3 into 4.2 yields:

\[
\frac{\partial C}{\partial t} = \kappa \left[ \frac{\partial^2 C}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \right]
\] (4.4)

Similar to chemical solutes, microbes migrate in porous media through diffusion-like Brownian motion. Such movement can be described using an equation analogous to the Fick’s second law (185):

\[
\frac{\partial X}{\partial t} = \frac{\tau \lambda_0}{R} \left[ \frac{\partial^2 X}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial X}{\partial r} \right) \right]
\] (4.5)

where \( \lambda_0 \) is the random motility coefficient for the microbes, with literature values from 2E-10 to 1.9E-9 m²/s for several species of bacteria (175, 185-186). Chemotaxis (i.e., movement of an organism in response to a chemical stimulus) was not evaluated in this study, therefore it is not considered in the model. Similar to the solutes evaluated in this study, values for \( \tau, \lambda_0 \) and \( R \) for the microbes were not evaluated separately but were lumped into a single parameter called the effective motility coefficient (\( \lambda_e \)), analogous to diffusivity (\( \kappa \)). The governing equation for microbial movement therefore becomes:

\[
\frac{\partial X}{\partial t} = \lambda_e \left[ \frac{\partial^2 X}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial X}{\partial r} \right) \right]
\] (4.6)

Diffusivities for compounds and microbes were estimated based on literature values for preliminary modeling of the intact rock core microcosms (Table 4-1). The values were allowed to vary during parameter optimization (section 4.4). The effective motility coefficient for microbes was assumed to be several orders of magnitude lower compared to the diffusivity of solutes, recognizing that microbes are bigger in size than
solute molecules and may attach to the solid phase, and tend to move more slowly in the rock matrix according to the Stokes-Einstein equation. While this parameter is likely to vary among different types of microbes, a single value was used in this study to simplify the modeling process.

For modeling scenarios in which a reaction or bacterial growth and decay occurs, a reaction term was added to equations 4.4 and 4.6:

\[
\begin{align*}
\frac{\partial C}{\partial t} &= \kappa \left[ \frac{\partial^2 C}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \right] + K/R \quad (4.7) \\
\frac{\partial X}{\partial t} &= \lambda_e \left[ \frac{\partial^2 X}{\partial z^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial X}{\partial r} \right) \right] + K/R \quad (4.8)
\end{align*}
\]

where \( K \) denotes the reaction kinetics of a chemical or growth and decay of bacteria (described in detail in the next two sections). \( R \) was set to 1 in the upper chamber of the microcosms and to greater than 1 within the rock, to account for the retardation effect caused by chemical adsorption or bacterial attachment. The reaction/retardation effect was applied to both biotic and abiotic transformations.

4.4.3 **Governing Equations: Biological Reductive Dechlorination**

 Monod kinetics was used for modeling reductive dechlorination (Eq. 4.9) and substrate limited microbial growth (Eq. 4.10):

\[
\frac{dC}{dt} = - \frac{\mu X}{Y} \frac{C}{K_s + C} = - \frac{\hat{q}XC}{K_s + C} \quad (4.9)
\]
\[
\frac{dX}{dt} = -\frac{dC}{dt} Y - d_H X = \frac{\hat{\mu} C}{K_S + C} X - d_H X = \frac{\hat{q} Y C}{K_S + C} X - d_H X \quad (4.10)
\]

where \( \hat{\mu} \) is the maximum specific growth rate, \( \hat{q} \) is the maximum specific substrate utilization rate, \( Y \) is the yield, \( K_S \) is the half-saturation coefficient, and \( d_H \) is the endogenous decay coefficient. For preliminary modeling, values for these parameters were estimated based on literature values (Table 4-1); they were allowed to vary during parameter optimization.

Based on results from the crushed rock microcosms, a lag period was expected prior to the onset of biotic reductive dechlorination of TCE. In preliminary modeling, an arbitrary lag time was used (Table 4-1). A term referred to as the “stall period” was also included, to capture events when the rate of TCE dechlorination slowed, possibly due to an inadequate supply of electron donor. To account for this, the maximum specific growth rate was replaced by an effective maximum rate:

\[
\hat{\mu}_{eff} = \hat{\mu}(1 - R_S), \quad \text{for} \quad t_{min} < t < t_{max} \quad (4.11)
\]

where \( \hat{\mu}_{eff} \) is the effective maximum specific growth rate, \( R_S \) is the stalling factor (0 \( \leq \) 1), and \( t_{min} \) and \( t_{max} \) define the period when the stall occurred.

### 4.4.4 Governing Equations: Abiotic Transformation

Abiotic transformation of TCE and cDCE was modeled as a pseudo first order process:

\[
\frac{dC}{dt} = -kC \quad (4.12)
\]
where \( k \) is the first order rate coefficient. Initial estimates for \( k \) were based on results from previous crushed rock microcosm study, as shown in Table 4-1.

Unlike biological reductive dechlorination, no lag time was considered for abiotic transformation of TCE and cDCE. Consequently, abiotic transformation of TCE started at time zero, and abiotic transformation of cDCE started once it was produced.

4.4.5 Governing Equations: Staple Isotope Transport and Reaction

Changes in \( \delta^{13} \text{C-TCE} \) and cDCE were modeled based on the kinetic isotope effect (KIE) (181, 187):

\[
KIE = \frac{k^l}{k^h} = \frac{1}{\left(1 + \epsilon\right)}
\]  

(4.13)

where \( k^l \) and \( k^h \) are rate constants for the light isotope and heavy isotopes, respectively, and \( \epsilon \) is the enrichment factor, indicating that the rate for the heavy isotope is slower than rate for the lighter isotope by an average of \( \epsilon \)‰.

For biological reactions that follow Monod kinetics, KIE depends on both the maximum specific growth rate (\( \hat{\mu} \)) and the half saturation coefficient (\( K_S \)) (Eq. 4.9 and 4.10). To simply this, it was assumed that KIE was affected only by \( \hat{\mu} \) (181), as expressed in equation 4.14:

\[
\frac{dC^i}{dt} = k^i C^i = -\frac{\hat{\mu}X}{Y} \cdot \frac{C^i}{K_S + C_T}
\]  

(4.14)

where \( C^i \) is the concentration of the \( i^{th} \) isotope (l for light, h for heavy), \( k^i \) is first order reaction coefficient for the \( i^{th} \) isotope, \( \hat{\mu}^i \) is maximum specific growth rate of bacteria on
the $i^{th}$ isotope, and $C_T$ is the total concentration of $^{12}$C- and $^{13}$C-molecules. Consequently, equation 4.13 can also be expressed as:

$$KIE = \frac{\bar{\mu}^l}{\bar{\mu}^h} = \frac{1}{(1 + \epsilon)} \quad (4.15)$$

KIE can also be applied to the diffusion coefficients:

$$KIE = \frac{D^l}{D^h} = \frac{1}{(1 + \epsilon)} \quad (4.16)$$

The relationships between the light and heavy isotopes for diffusion, abiotic degradation, and biological reductive dechlorination are:

$$D^h = D^l (1 + \epsilon_{\text{diff}}), \quad \text{for diffusion} \quad (4.17)$$

$$k^h = k^l (1 + \epsilon_{\text{abio}}), \quad \text{abiotic degradation} \quad (4.18)$$

$$\bar{\mu}^h = \bar{\mu}^l (1 + \epsilon_{\text{bio}}), \quad \text{for biotic degradation} \quad (4.19)$$

In the model, TCE molecules with different isotopic composition (i.e., $^{12}$C-TCE and $^{13}$C-TCE) were treated as separate species (187). Their respective proportion in total TCE can be calculated based on initial $\delta^{13}$C-TCE. This number represents the ratio of heavy isotope to light isotope $(R = ^{13}$C$_{\text{TCE}}^{/}$^{12}$C_{\text{TCE}} )$ in the sample over the ratio in a standard, i.e.,

$$\delta^{13}C_{\text{TCE}} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \%_o \quad (4.20)$$
Based on the international standard for the carbon stable isotope ratio \( R_{\text{standard}} = 0.0112372 \), determined based on Vienna Pee Dee Belemnite) and the initial total TCE concentration \( (C_{\text{TCE,o}}) \), the starting concentrations for \(^{12}\text{C}-\text{TCE} \) and \(^{13}\text{C}-\text{TCE} \) were calculated as follows:

\[
^{12}C_{\text{TCE,o}} = \frac{C_{\text{TCE,o}}}{\delta^{13}C_{\text{TCE,o}} + 1000} \cdot R_{\text{reference}} \tag{4.21}
\]

\[
^{13}C_{\text{TCE,o}} = \frac{C_{\text{TCE,o}}}{1 + \frac{1000}{\delta^{13}C_{\text{TCE,o}} + 1000}} \cdot R_{\text{reference}} \tag{4.22}
\]

Initial concentrations \( (^{12}C_{\text{TCE,o}}, ^{13}C_{\text{TCE,o}}) \) and model parameters were assigned, as shown in Table 4-1. By simulating the temporal and spatial changes for \(^{12}\text{C}_{\text{TCE}}, ^{13}\text{C}_{\text{TCE}}, \) and their respective daughter products \( (^{12}\text{C}_{\text{cDCE}} \) and \(^{13}\text{C}_{\text{cDCE}} \)), the model simultaneously determined the change in total \( C_{\text{TCE}} \) (the sum of \(^{12}\text{C}_{\text{TCE}} \) and \(^{13}\text{C}_{\text{TCE}} \)) and total \( C_{\text{cDCE}} \) (the sum of \(^{12}\text{C}_{\text{cDCE}} \) and \(^{13}\text{C}_{\text{cDCE}} \)), as well as \( \delta^{13}C_{\text{TCE}} \) and \( \delta^{13}C_{\text{cDCE}} \) (based on equation 4.20).

A starting value of -0.2‰ was assigned for diffusion of TCE \( (\varepsilon_{\text{TCE,diff}}) \) and cDCE \( (\varepsilon_{\text{DCE,diff}}) \) (Table 4-1), based on Wanner et al. (174). For abiotic transformation \( (\varepsilon_{\text{abio}}) \), a temporary value of -25‰ was assigned to TCE \( (\varepsilon_{\text{TCE,abio}}) \) and cDCE \( (\varepsilon_{\text{DCE,abio}}) \) (Table 4-1), based on a range of values from the literature (Tables 1-1 to 1-3). For reductive dechlorination of TCE to cDCE \( (\varepsilon_{\text{bio}}) \), a starting value of -15‰ was assigned to \( \varepsilon_{\text{TCE,bio}} \) (Table 4-1) based on the literature (Tables 1-2 and 1-3) and an assumption that the enrichment factor for biotic reductive dechlorination is less negative than for abiotic transformation (79). This is also in line with reported values for biodegradation of TCE.
by *Geobacter* (-7.9 to -12.7‰) (84, 86-87, 181) and by a *Geobacter*-containing microbial consortium (-2.5 to -14.5‰) (70-71).

Parameters in Table 4-1 are grouped according to their roles in different processes:

- **Group 1** ($T$ to $\delta^{13}C_{TCE,o}$) comprises basic characteristics of the intact core microcosms (e.g., rock porosity, TCE concentration);
- **Group 2** ($\kappa_{Br}$ to $\varepsilon_{DCE,diff}$) comprises parameters associated with diffusion;
- **Group 3** ($R_s$ to $T_{lag}$) comprises biodegradation; note that “N/A” (not applicable) is indicated for three of the parameters related to the occurrence of a stall in TCE reductive dechlorination and are therefore not generally applicable;
- **Group 4** ($k_{TCE}^f$ to $\varepsilon_{DCE,abio}$) comprises abiotic process; and
- **Group 5** ($R$) comprises retardation factor of reaction in rock matrix.

### 4.5. Model Implementation

#### 4.5.1 Preliminary Modeling

Modeling was initially performed for six scenarios that encompass different reactive and transport processes (Appendix C.2). A comparison between the experimental data and the six scenarios indicated that the behavior of microcosms U1, U3, U5, U6 and L3 matches the simulation scenarios where only diffusion and abiotic degradation of TCE occurs, hereafter referred to as scenario I; the behavior of L1, L2, L4, L5, L6, and U2 is best represented by a combination of diffusion, reductive dechlorination of TCE and abiotic degradation of TCE and cDCE, hereafter referred to as scenario II.
4.5.2 Model Calibration

Parameter optimization was conducted on individual core microcosms and final values are summarized in Table 4-2. The parameters that were altered in simulation scenario I included $\kappa_{Br}$, $\kappa^l_{TCE}$ and $k^l_{TCE}$ (and consequently $t_{1/2,TCE}$); the parameters altered in scenario II included $\kappa_{Br}$, $\kappa^l_{TCE}$, $\kappa^l_{DCE}$, $\mu_{max}$, $T_{lag}$, $k^l_{TCE}$ (also $t_{1/2,TCE}$), $k^l_{DCE}$ (also $t_{1/2,DCE}$), and $\varepsilon_{DCE,abio}$.

Representative simulation results are shown in Figures 4-2 and 4-3 for intact core microcosm pair #5; panel a presents the results for bromide; panel b for TCE and cDCE; and panel c for $\delta^{13}$C. Additional model simulations for the remaining microcosms may be found in Appendix C.3. The model reasonably matches the data. Although concentration data within the cores were not measured over time, model predictions for TCE, cDCE, $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE are shown.

Diffusivities for TCE in all microcosms except pair #1 ranged from 1.2E-12 to 1.9E-12 m$^2$/s, comparable to two of the three values for TCE from other reports involving sandstone from the studied site (172-173), and approximately an order of magnitude lower than those reported or calculated for Cl$^-$, MnO$_4$\(^-\) and HTO (171, 184) (Table 4-3). The diffusivities for bromide, TCE and cDCE in pair #1 were an order of magnitude lower than those for the other pairs, consistent with the lower head chamber concentrations observed in pair #1 (Appendix C.3). The diffusivity for TCE in pair 1 (1.2E-13 to 1.9E-13 m$^2$/s) are close to the value (1.2E-13 m$^2$/s) in one study that predicted a very low tortuosity and a high retardation factor (172).
Table 4-2. Summary of finalized modeling parameters.\(^a\)

| Parameter   | units | Scenario I |         |         |         |         |         |         |         |         |         | Scenario II |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|-------------|-------|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| \(\kappa_{Br}\) | m\(^2\)/s | 2.0E-12 | 2.5E-11 | 2.5E-11 | 2.5E-11 | 2.5E-11 | 2.0E-12 | 2.5E-11 | 2.5E-11 | 2.5E-11 | 2.5E-11 | 2.5E-11 |
| \(k'_{TCE}\) | m\(^2\)/s | 1.2E-13 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 | 1.9E-12 |
| \(k'_{DCE}\) | m\(^2\)/s | N/A\(^b\) | N/A | N/A | N/A | N/A | 1.3E-12 | 8.8E-12 | 1.3E-11 | 2.2E-11 | 1.3E-11 | 1.6E-11 |
| \(R_s\) | dim\(^c\) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| \(t_{\text{min}}\) | s | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 6.6E+06 | 1.6E+07 | N/A | 8.5E+06 |
| \(t_{\text{max}}\) | s | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 6.6E+06 | 1.6E+07 | N/A | 8.5E+06 |
| \(\mu'_{\text{max}}\) | s\(^{-1}\) | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 9.0E-07 | 9.0E-07 | 9.5E-07 | 8.5E-07 | 9.0E-07 | 9.3E-07 |
| \(T_{\text{lag}}\) | s | N/A | N/A | N/A | N/A | N/A | 1.7E+07 | 3.6E+06 | 2.1E+06 | 6.8E+06 | 6.5E+06 | 3.0E+06 |
| \(T_{\text{lag}}\) | s | N/A | N/A | N/A | N/A | N/A | 1.7E+07 | 3.6E+06 | 2.1E+06 | 6.8E+06 | 6.5E+06 | 3.0E+06 |
| \(k_{1/2,TCE}\) | s\(^{-1}\) | 5.0E-10 | 3.0E-10 | 4.4E-10 | 5.4E-10 | 3.6E-10 | 5.0E-10 | 6.0E-10 | 5.0E-10 | 3.5E-10 | 3.5E-10 | 2.5E-10 |
| \(t_{1/2,TCE}\) | yr | 44 | 74 | 50 | 41 | 61 | 44 | 37 | 44 | 63 | 63 | 88 |
| \(k_{1/2,DCE}\) | s\(^{-1}\) | N/A | N/A | N/A | N/A | N/A | 3.5E-10 | 3.5E-10 | 3.5E-10 | 6.0E-10 | 6.0E-10 | 4.5E-10 |
| \(t_{1/2,DCE}\) | yr | N/A | N/A | N/A | N/A | N/A | 63 | 63 | 63 | 37 | 37 | 49 |
| \(\varepsilon_{\text{DCE,abio}}\) | dim | N/A | N/A | N/A | N/A | N/A | -20\(\%\) | -20\(\%\) | -20\(\%\) | -20\(\%\) | -20\(\%\) | -20\(\%\) |

\(^a\) Values in bold are changed from the preliminary ones in Table 4-1. For parameters that are not listed, no changes were made to the preliminary ones.

\(^b\) N/A = not applicable to this microcosm.

\(^c\) dim = dimensionless.
Figure 4-2. Numerical model fit of (a) bromide, (b) TCE, and (c) δ^{13}C for intact rock core microcosm U5.
Figure 4-3. Numerical model fit of (a) bromide, (b) TCE and cDCE, and (c) $\delta^{13}C$ for intact rock core microcosms L5.
Monod parameters were consistent with the range of values found in the literature for reductive dechlorination of TCE by *Geobacter* and/or *Dehalococcoides* (Table 4-4). The rates obtained for abiotic transformation of TCE and cDCE were lower compared to those reported for pure minerals (Table 1-4) and those based on previous crushed rock and intact core studies (Appendix C.4). This is further discussed in section 4.5.4.

### 4.5.3 Mass Balances and Goodness of Fit

Goodness of fit was evaluated by comparing the measured and model-simulated mass balances for bromide and total chlorinated ethene removal (Fig. 4-4). Excluding pair #1, the average total measured amount of bromide (removed + remaining in the core; yellow triangles in Fig. 4-4a) was 15.5±7.8% (α=0.05) lower than the model prediction (blue + yellow bars in Fig. 4-4a), indicating the model was reasonably close to the data.

The model prediction of initial bromide mass was based on an assumption of uniform saturation and 13% porosity. For pair #1, there was reasonably good agreement between the measured bromide removed (blue triangle in Fig. 4-4a) and the model prediction (blue bar in Fig. 4-4a); however, there was a larger difference in the total measured amount of bromide and the model prediction, likely because the porosity of pair #1 is lower than 13%, and therefore the estimate of initial bromide level was too high.

Data for the mass of chlorinated ethenes remaining in the core was not available, so total recovery was not calculated. Comparisons were therefore made between the measurement of mass removed and the model prediction. The average difference between the measured TCE + cDCE removed (green triangles in Fig. 4-4b) and the model
Table 4-3. Comparison of diffusivity and diffusion coefficient obtained from this study or reported by literatures.

<table>
<thead>
<tr>
<th>Compound (pair)</th>
<th>$\kappa$ ($\times 10^{-12} \text{ m}^2/\text{s}$)</th>
<th>$D_F$ ($\times 10^{-9} \text{ m}^2/\text{s}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>This study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCE (pair 1)</td>
<td>0.12 – 0.19 $^a$</td>
<td>0.91 (183)</td>
</tr>
<tr>
<td>TCE (pairs 2 – 6)</td>
<td>1.2 – 1.9 $^a$</td>
<td>0.91</td>
</tr>
<tr>
<td>cDCE (pair 1)</td>
<td>1.3 $^a$</td>
<td>1.13 (183)</td>
</tr>
<tr>
<td>cDCE (pairs 2 – 6)</td>
<td>8.8 – 22 $^a$</td>
<td>1.13</td>
</tr>
<tr>
<td>Br$^-$ (pair 1)</td>
<td>2.0 $^a$</td>
<td>1.9 (182)</td>
</tr>
<tr>
<td>Br$^-$ (pair 2 – 6)</td>
<td>25 $^a$</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Site literature</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>12 – 37 (171) $^b$</td>
<td>1.6 (188)</td>
</tr>
<tr>
<td>MnO$_4^-$</td>
<td>7 – 12 (184)</td>
<td>1.6 (189)</td>
</tr>
<tr>
<td>HTO</td>
<td>6 – 38 (184)</td>
<td>2.2 (190)</td>
</tr>
<tr>
<td>TCE</td>
<td>4.3 (173) $^b$</td>
<td>1.0 (173)</td>
</tr>
<tr>
<td></td>
<td>0.12 (172)</td>
<td>0.86 (191)</td>
</tr>
<tr>
<td></td>
<td>6.5 (172) $^b$</td>
<td>1.0 (172)</td>
</tr>
</tbody>
</table>

$a$ Value was obtained through parameter fitting.

$b$ Value was not directly reported in the literature but calculated based on equation 4.3.
Table 4-4. Monod parameters used in the model and compared to the literature on dechlorinators.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range of Values</th>
<th>References</th>
<th>Converted to units used in this model$^a$</th>
<th>Optimized values from this model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{\text{TCE}}$</td>
<td>0.25 – 17.9 (mg VSS/mmol TCE)$^a$</td>
<td>(139, 175-180)</td>
<td>$1.4 \times 10^6 – 1.0 \times 10^{10}$ (copies/mg TCE)</td>
<td>$2.0 \times 10^9$ (copies mg/TCE)</td>
</tr>
<tr>
<td>$q_{\text{max}}$</td>
<td>0.75 – 2,222 (μmol TCE/mg VSS/d)</td>
<td>(175-178, 180)</td>
<td>$1.5 \times 10^{-17} – 4.6 \times 10^{-14}$ (mg TCE/copy/s)</td>
<td>$4.5 \times 10^{-16}$ (mg TCE/copy/s)</td>
</tr>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>0.25 – 1.43 (1/d)</td>
<td>(175-178, 180)</td>
<td>$2.8 \times 10^{-6} – 1.7 \times 10^{-5}$ (1/s)</td>
<td>$0.9 \times 10^{-6}$ (1/s)</td>
</tr>
<tr>
<td>$K_S$</td>
<td>0.08 – 4.20 (μM TCE)</td>
<td>(175-178, 180)</td>
<td>0.01 – 0.55 (mg TCE/L)</td>
<td>0.07 (mg TCE/L)</td>
</tr>
<tr>
<td>$d_H$</td>
<td>0.03 – 0.06 (1/d)</td>
<td>(175, 177)</td>
<td>$3.6 \times 10^{-7} – 7.3 \times 10^{-7}$ (1/s)</td>
<td>$4 \times 10^{-7}$ (1/s)</td>
</tr>
</tbody>
</table>

$^a$ VSS = volatile suspended solids.
Figure 4-4. Comparison of measured and model simulated (a) bromide recovery and (b) total chlorinated ethene removal. Modeling results are shown in bars and measured results are shown in triangles. Measured bromide remained in (a) is actually “removed + remained,” measured cDCE in (b) is actually “DCE + TCE”; both indicated by an asterisk (*) in the legends.
predicted TCE + cDCE removed (red + green bar in Fig. 4-4b) was 2.6±1.2% (α=0.05). These reasonably good fits validate the assumption used in the model that the core was well saturated and the compounds were homogeneously distributed.

A similar analysis was not possible for the δ\textsuperscript{13}C data. Nevertheless, the model fits in Figures 4-2c and 4-3c reasonably represent the trends. In a few of the microcosms (Appendix C.3), enrichment tended to level off, which was not captured by the model since a first order reaction rate was assumed. This is further discussed in section 4.5.5.

### 4.5.4 Sensitivity Analysis

Sensitivity analysis was performed to investigate the effect of varying a single parameter on the overall model fit. The parameters evaluated were sampling removal rate, microbial motility, chemical diffusivities, abiotic transformation rates, enrichment factors, reaction retardation factor, and Monod kinetics (Appendix C.5). Results indicated that Monod parameters (except for yield) and the reaction retardation factor (R) have significant impacts on both VOC and isotope simulations. The sampling removal rate (\(k_{chamber}\)) and chemical diffusivities (\(κ\)) greatly influence bromide and VOC but not isotope enrichment. Abiotic transformation rate constants (\(k\)) and biotic and abiotic enrichment factors (\(ε\)) have significant impacts on isotope simulation but little or no effect on VOCs. Yield (\(Y\)) and microbial motility (\(λ_e\)) have little or no impact on all the model fits.

Sensitivity analysis showed that the enrichment factor (\(ε\)) and rate constant (\(k\)) for abiotic transformation have little impact on the VOC profile but are proportionally related to the enrichment rate of δ\textsuperscript{13}C (Appendix C.5). As a result, the optimal value for one
parameter is highly dependent on the value of the other. In this model, the enrichment factors for abiotic transformation were allowed to vary within the broad range reported in the literature, i.e., -7.1‰ to -51.0‰ for TCE and -4.9‰ to -23.5‰ for cDCE. Site-specific values were not available and may be outside this range. Since rate constants are of specific interest for site remediation, a sensitivity analysis was conducted to evaluate the influence of the enrichment factor on rate estimation.

Enrichment factors were varied over the reported range and the corresponding rate that achieved the optimal δ^{13}C fit in each microcosm was estimated. Half-lives are plotted against the enrichment factors rather than the rates (Fig. 4-5). The magnitude of the enrichment factor has a significant impact: The less negative the enrichment factor, the shorter the half-life (i.e., faster rate); the relationship is almost inversely proportional. For example, a TCE abiotic enrichment factor of -33.4‰, as reported for FeS at pH 8 (77), resulted in TCE half-lives from 55 to 100 yr, while an enrichment factor of -39‰, reported for magnetite (77), led to half-lives of 67-122 yr. As for cDCE, a mid-range abiotic enrichment factor of -15‰ corresponds to cDCE half-lives of 25-44 yr, while the highest abiotic enrichment factor reported for cDCE, -23.5‰ (64), led to half-lives of 42-69 yr. For “typical” enrichment factors of -25‰ for TCE and -20‰ for cDCE, the resulting half-lives are 37-88 yr for TCE and 37-63 yr for cDCE (Table 4-2). This level of uncertainty can be narrowed by using site-specific enrichment factors.

The transformation rates estimated from the intact core microcosm are longer than those determined with crushed rock microcosms based on the rate of ^14C-product accumulation (14-25 yr for TCE and 11-31 yr for cDCE; Chapter 2). This may be a
inversely proportional. For example, a TCE abiotic enrichment factor of -33.4‰, as reported for FeS at pH 8 (77), resulted in TCE half-lives from 55 to 100 yr, while an enrichment factor of -39‰, reported for magnetite (77), led to half-lives of 67-122 yr. As for cDCE, a mid-range abiotic enrichment factor of -15‰ corresponds to cDCE half-lives of 25-44 yr, while the highest abiotic enrichment factor reported for cDCE, -23.5‰ (64), led to half-lives of 42-69 yr. For “typical” enrichment factors of -25‰ for TCE and -20‰ for cDCE, the resulting half-lives are 37-88 yr for TCE and 37-63 yr for cDCE (Table 4-2). If site-specific enrichment factors were available, the uncertainty could be narrowed considerably.

The transformation rates estimated from the intact core microcosm are longer than those determined with crushed rock microcosms based on the rate of 14C-product accumulation (11-21 years for TCE and 11-31 years for cDCE; Chapter 2). Crushing increases the surface areas of the active minerals. Surface-area normalized rate constants may allow for a more robust comparison.

Figure 4-5. The sensitivity of (a) the abiotic TCE degradation rate (in the form of half-life) for scenario I to the full range of reported values for abiotic enrichment factor of TCE; and (b) the sensitivity of the abiotic cDCE degradation rate (in the form of half-life) for scenario II to the full range of reported values for abiotic enrichment factor of cDCE. Shaded areas represent the half-lives used in final model simulation, corresponding to enrichment factors of -25‰ for TCE and -20‰ for cDCE.
consequence of an increase in the surface area of the active minerals in crushed rock. Surface-area normalized rate constants may allow for a more robust comparison.

Another issue evaluated by sensitivity analysis is where degradation activities take place (i.e., in the head chamber, within the rock, or both). Simulations were performed to check the model’s response to the absence of biotic or abiotic activity in either the head chamber or the core section. Four variations of scenario I were simulated, in which abiotic transformation of TCE occurred in the chamber, the core, neither, or both (Fig. 4-6, panels a and b). The profile for TCE was slightly higher when reaction was absent in the core, while there was almost no change when reaction was absent in the chamber, probably because the majority of TCE mass resides in the core. As for δ^{13}C-TCE, the absence of abiotic transformation in the chamber had little impact on δ^{13}C-TCE enrichment, but the absence of reaction in the core greatly reduced the extent of δ^{13}C-TCE enrichment. This is potentially because only a small level of δ^{13}C-TCE enrichment occurred in the chamber, which was diminished by weekly sample removal and diffusion of TCE out of the core into the chamber (i.e., a δ^{13}C-TCE depletion process for the chamber). Similar trends were observed in the core section (not shown). This sensitivity analysis suggests that abiotic transformation is governed by reactions in the core.

Variations for scenario II, in which TCE is reduced to cDCE and both undergo abiotic transformation are shown in Figures 4-6, panels c, d, and e. Abiotic transformation was maintained in the chamber and the core for this simulation. Three cases were simulated in this sensitivity analysis, including reductive dechlorination in the core, in the chamber, or both. The absence of reductive dechlorination in the core section
Figure 4-6. Sensitivity of model to (a and b) the absence of abiotic transformation in chamber or core in scenario I; and to (c-f) the absence of biological reductive dechlorination in chamber or core in scenario II. Only the simulation results for the chamber are shown, except in (e), which represents the VOC change in the core.
has the biggest impact on VOCs in the chamber. TCE transformation slowed the most, and there is a rebound after it hits the lowest level. This lasted through the rest of the simulation period, possibly due to continuous diffusion of TCE from the core into the chamber. The increase in cDCE in the chamber was slow and the maximum cDCE concentration was much lower compared to that of TCE, because cDCE was removed through sampling and diffusion into the core. The absence of reductive dechlorination in the chamber had an interesting effect: TCE was almost unaffected, indicating the drop in TCE in the chamber is primarily driven by degradation inside the core; however, the increase in cDCE followed an initial increase, then a slight drop, another rise to a maximum level, and finally a continuous decrease. An examination of the concentration profile in the core showed that the full onset of reductive dechlorination was delayed when the reaction was restricted to the core (Fig. 4-6e). The reason for this behavior is unclear but is likely related to the low biomass level in the core. $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE enrichment in the chamber was delayed by the absence of reductive dechlorination in the core, similar to the response of TCE and cDCE (Fig. 4-6d). The absence of reductive dechlorination in the chamber caused an unexpected pattern (Fig. 4-6f). Comparing the sensitivity results to experimental data from the core microcosms indicated that reductive dechlorination occurred in both the chamber and the core.

4.5.5 Model Deviations and Implications

Even though the model fit reasonably well to the experimental data, several deviations were noted, including: 1) Model simulation of the diffusion process and sampling removal appears to generate a “smoother” bromide and TCE curve at the very
beginning, compared to the rather sharp rise and drop of the actual data (Fig. 4-2 and 4-3). This pattern occurred in both the continuous and weekly models (Appendix C.1) therefore it does not appear to be a consequence of using a simplified sampling simulation. One potential reason is an imperfect initial condition, such as TCE and bromide residual being trapped in dead zones inside the head chamber during flushing. An unexpected concentration spike may have occurred after several weeks of equilibration. This may also be caused by adsorption of TCE to the rock matrix or even the Teflon wrapping, which could start desorbing immediately after head chamber flushing. However, the non-absorbable bromide tracer displayed a similarly “sharp” pattern as TCE, indicating that adsorption may not play a major role in this modeling deviation. Another potential reason is the presence of air bubbles in the core as a result of insufficient initial saturation. TCE diffusivity is much higher in the gas phase than in water. The overall effect would be an increase in TCE bulk diffusivity and a large TCE flux from the core to the chamber, which led to a concentration spike in the chamber. The air bubbles either dissolve or move up the core through interconnected pores and ultimately enter the head chamber. This would decrease the bulk diffusivity of TCE in the core and cause a prominent tailing effect.

2) Some small fluctuations in data were not captured by the model, particularly, deviations in $\delta^{13}$C enrichment at the later stages of monitoring. These changes may reflect processes not included in the model, such as exhaustion of mineral capacity for abiotic transformation. Since available data did not offer sufficient information to identify
the actual reason, no effort was made to calibrate for these deviations. The goal here is to keep the model as simple as possible, as long as it captures the overall trends.

3) Close to the end of the incubation, TCE appears to decrease more slowly than the model prediction, while bromide simulation matches the data relatively well. This suggests the deviation was likely caused by TCE adsorption. For instance, decreasing the aqueous TCE concentration in the pore spaces due to back diffusion from the matrix to the head chamber would enhance desorption of TCE from the solid phase, which in turn would sustain the TCE concentration in the pore spaces and magnify the tailing effect. Adsorption affected cDCE simulation to a much lesser extent because there was no “pre-absorbed” cDCE at the beginning of the experiment. Inclusion of adsorption and desorption in the model may improve the fit for TCE.

4) For convenience purposes, the simulation of weekly sampling was substituted by a continuous decay process with a pseudo-first order rate. This simplification assumed complete mixing in the head chamber, while in reality equilibrium may take longer to reach after each sampling event. Potential consequences of this simplification have not yet been fully evaluated. Another issue worthy of attention is the difference between the estimated removal rates for bromide (2.3E-7 s^{-1}) and that for TCE, cDCE, and microbes (1.7E-7 s^{-1}), which could be related to retardation effects (e.g., absorption and attachment).

4.6. Summary and Recommendations

The model developed in this study combined Monod kinetics, first order kinetics, Fick’s law of diffusion, and kinetic isotope effects to describe the processes observed in
intact rock core microcosms. The model was calibrated with experimental data and achieved good fits and reasonable mass balances.

Sensitivity analysis indicated that several parameters may play key roles in predicting TCE and cDCE transport and reaction. These parameters include matrix porosity, maximum specific growth rate, decay coefficient, and diffusivity. Also, the values used for the enrichment factor for abiotic transformation of TCE and cDCE have a significant impact on estimation of the rate constants. Consequently, site-specific enrichment factors are required to get more precise estimates of the rates.

Additional model simulations indicate that abiotic transformation is governed by reactions in the core, while reductive dechlorination occurred in both the chamber and the core. The existence of an impermeable block that occupied 20% of the rock porosity did not significantly alter the modeling results, indicating that the presence of a low level of unsaturation does not significantly alter the behavior of the microcosms. Still, a better understanding of the initial saturation level, compound distribution, and the effect of adsorption, is expected to yield more precise model predictions.

Abiotic transformation rates predicted by the model based on intact rock core microcosms were lower than those determined with $^{14}$C-labeled compounds in the crushed rock microcosms. The crushing process likely results in overestimated rates due to the greater surface area; surface area normalized rates may be more useful for comparison. Nevertheless, the rates determined from the intact rock core microcosms, corresponding to half-lives of 37 to 88 years for TCE (based on an enrichment factor of -25‰) and 37 to 63 years for cDCE (based on an enrichment factor of 20‰), are
meaningful in the context of diffusion-controlled restoration time-scales and plume stability at this site.

Overall, this model served as a useful tool to interpret data from intact rock core microcosms. Most importantly, it enabled the analysis of individual processes within an interactive system, and permitted parameter estimation which expands the applicability of the laboratory experimentation to field practice. The model is a valuable addition to the novel intact core microcosm method to study fractured bedrock and other heterogeneous/low permeability media.
5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Three approaches were employed in this study to evaluate natural and enhanced attenuation of chlorinated ethenes inside a fractured bedrock aquifer. The first two approaches were laboratory studies employing two different types of microcosms, one constructed with crushed rock and the other with intact cores following a novel design. The advantage of crushed rock microcosms lies in their simplicity and versatility, but since the rock is crushed and homogenized, the degree to which it is representative of in situ conditions is questionable. Intact rock core microcosms, on the contrary, are tricky to setup and the data are not always easily interpretable; on the positive side, they faithfully preserve the matrix integrity and therefore the diffusion process. The novel intact rock core microcosms developed in this study performed consistently, and the results from both types of microcosms are in reasonable agreement with each other and with field observations; minor differences existed but are explainable. Thereby, this novel method is deemed a viable approach to replicate contaminant transport and reaction in dual-porosity media with the presence of a low permeability zone. And the design principles and techniques may be easily transferred to simulation of other complicated geological settings. The third approach in this study used numerical modeling to simulate the coupled effects of diffusion, reaction, and sampling in the intact core microcosms. Adequate fits to the data validated the accuracy of the model; furthermore, prediction of transformation rates was achieved via parameter optimization. These results suggested
that intact core microcosms and a core-scale model can be integrated for more realistic and accurate evaluation of remediation strategies.

The specific conclusions from this study are:

1) Reductive dechlorination of TCE to cDCE occurred in unamended controls of both crushed rock microcosms and intact core microcosms. This is consistent with previous laboratory studies and field observations of site wide cDCE occurrence, suggesting the existence of indigenous dechlorinators and microbially-mediated natural attenuation.

2) Addition of three types of substrates (i.e., lactate, EVO and HRC) enhanced the rate and extent of TCE reduction to cDCE in crushed rock microcosms, indicating that the indigenous chlororespiring bacteria are starved for electron donor and are readily stimulated by amendments.

3) Lactate further stimulated reductive dechlorination of cDCE to VC and ethene in crushed rock microcosms, suggesting the in situ presence of *Dehalococcoides*, which are the only known genera of bacteria capable of reductively dechlorinating cDCE and VC to ethene (*Dehalogenimonas* have been shown to respire tDCE and VC to ethene). The fact that VC or ethene has only been detected at trace levels in situ indicates that the *Dehalococcoides* population is low and the redox conditions or substrate availability cannot support significant growth. The reason that EVO and HRC did not simulate reduction beyond cDCE may be due to their slow fermentation rates; i.e., they did not produce hydrogen at a high enough rate to sustain the activity of *Dehalococcoides*. 

-142-
4) Genetic analyses of crushed rock microcosms and enrichment cultures demonstrated that *Dehalococcioides* are responsible for cDCE reduction to VC and ethene, while a *Geobacter* sp. likely performs reduction of TCE to cDCE. *Geobacter* are ubiquitous in the subsurface, tolerate higher redox levels than *Dehalococcioides*, and are capable of using ferric iron as an electron acceptor with a variety of electron donors. It is not surprising that *Geobacter* are involved in reduction of TCE to cDCE across the site.

5) VC reduction to ethene was slower compared to TCE and cDCE reduction and represents the rate limiting step for complete reductive dechlorination. Genetic analysis revealed that *D. mccartyi* is closely related to the *Dehalococcioides* sp. at this site. Several strains of *D. mccartyi* are known to metabolically respire cDCE but co-metabolically degrade VC, as opposed to other strains that perform both reactions metabolically. Since VC is a known human carcinogen, the risk of transient accumulation of VC should be weighed carefully when evaluating biostimulation for this site.

6) A simplified fracture-matrix microcosm was constructed using sections of intact core. Intact rock is expected to better represent in situ processes, particularly matrix diffusion. The prototype developed in this study successfully addressed design challenges related to microcosm setup, sample collection, and data interpretation. A leak-proof seal was achieved via layers of Teflon tape, heat-shrinkable Teflon tubing, and a stainless steel pipe welded on the ends to stainless steel caps. Exchange of water in the simulated fracture zone was made weekly.
through two sampling valves, at a controlled flow rate to minimize unwanted mixing. This exchange also permits sample collection and donor delivery. The intact core microcosms were monitored for almost two years and the behavior was consistent with expectations for a diffusion dominated system. Using numerical modeling to facilitate data analysis greatly enhanced the value of this experiment.

7) Lactate addition increased the frequency and rate of TCE reduction to cDCE in the intact core microcosms, similar to what was observed in crushed rock microcosms. More than half of the lactate-amended crushed rock microcosms exhibited additional VC and ethene production, which did not occur in any of the intact core microcosms. This indicated an absence of *Dehalococcoides* in the cores used to construct the intact rock microcosms, which is reasonable given the likely heterogeneous distribution of *Dehalococcoides* at this site. It is also possible that the initial concentration of TCE within the cores (~20 mg/L) inhibited the growth of *Dehalococcoides*.

8) Cores that exhibited reductive dechlorination of TCE to cDCE experienced two-fold higher removal of total chlorinated ethenes, implying a potential benefit of biostimulation. Increased removal was presumably a consequence of an increased concentration gradient between the fracture and the matrix, and the fact that cDCE diffuses at a higher rate and adsorbs less in comparison to TCE.

9) Transformation of TCE and cDCE by processes other than reductive dechlorination was confirmed by enrichment in $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE in the crushed rock and intact core microcosms, as well as accumulation of $^{14}$CO$_2$ and
10) Addition of electron donor or donor plus sulfate to the crushed rock microcosms promoted sulfate reducing conditions but did not enhance transformation processes other than reductive dechlorination. It is possible that the site geochemistry and mineralogy are not amenable to generation of new minerals, or there are other factors that limit abiotic transformation. Instead, sulfate reduction appeared to compete with biotic reductive dechlorination for electron donors, and sulfide may have reacted with reducing minerals and decreased their abiotic transformation capacity.

11) The pseudo-first order transformation rates for TCE and cDCE in crushed rock microcosms were 0.038 and 0.044 yr⁻¹, corresponding to half lives of 18 and 16 yr, respectively. They were estimated based on accumulation of \(^{14}\)CO\(_2\) and \(^{14}\)C-NSR. Uncertainties created by rock crushing and homogenization on abiotic and biotic reactions need to be further assessed.

12) Intact core microcosms avoid the uncertainties created by rock processing. However, their inherit complexity limits the number of treatments that can be evaluated and the volume of samples available for analysis. For this study, estimates of TCE and cDCE transformation rates by processes other than reductive dechlorination in the intact core microcosms was limited to model
simulations of δ^{13}C enrichment data. Uncertainties in enrichment factors led to wide ranges in estimates of the transformation rates: 0.008-0.019 yr^{-1} for TCE and 0.011-0.019 yr^{-1} for cDCE, corresponding to half lives of 37-88 yr for TCE and 37-63 yr for cDCE. Longer half-lives compared to crushed rock microcosms may be attributable to the increased mineral surface area created by crushing. Nevertheless, the half lives based on intact core microcosms are significant in the context of diffusion-controlled restoration time-scales and plume stability at this site.

13) Additional studies revealed that lactate amendment results in acetate and hydrogen formation. Sulfate reduction was supported by lactate and hydrogen but not acetate, while reductive dechlorination of TCE to cDCE and VC were supported by all three electron donors. Acetate is likely to support cDCE to VC reduction via its oxidation to CO_{2} and hydrogen, suggesting that acetate in the intact core microcosms was beneficial. As the end-of-incubation analysis showed, acetate diffused significantly into the intact core microcosms, so that electron donor was available for reductive dechlorination throughout the core, not just in the groundwater in the simulated fracture.

14) Model sensitivity analysis indicated that several parameters may play key roles in predicting TCE transport and reaction, including effective diffusivity, maximum specific growth rate, and decay coefficient. Further sensitivity analysis indicated that abiotic transformation is governed by reactions in the core, and reductive dechlorination occurred in both the chamber and the core. Reducing the level of
core saturation by 20% lessened the predicted concentration in the head chamber
but did not have a substantial impact on the overall trends.

Overall, this study provides several insights into both natural and biostimulated
attenuation of chlorinated ethenes at a fractured bedrock site. The information gained
suggests that reductive dechlorination of TCE to cDCE, which is a site wide process, can
be further enhanced via biostimulation. Complete TCE reduction to cDCE may enhance
the transport of contaminant mass from the rock matrices to the fractures, thereby making
them more available for other remedies and shortening the remediation timeline. The
abiotic-mediated portion of natural attenuation is occurring at rates relevant for plume
control and long term mass destruction. However, the rate and capacity appeared to vary
with rock type and method employed to make the measurement (i.e., crushed versus
intact rock). Recommendations for improvements to future research are listed in the next
section.
5.2. Recommendations

Although the objectives of this study were met, several improvements are recommended with respect to materials, analytical methods and experimental design, many of which pertain to the novel intact core microcosms:

1) In crushed rock microcosms with $^{14}$C-TCE added, autoradiolysis of $^{14}$C-TCE appeared to have occurred in the distilled deionized water controls while being quenched in bottles containing groundwater. Autoradiolysis of $^{14}$C-TCE led to the production of a high background of $^{14}$CO$_2$ and $^{14}$C-NSR in the water controls, which complicated interpretation of results in the microcosms. Future studies should employ filter sterilized groundwater as water controls. The presence of alkalinity and natural organic matter in the groundwater mitigates the impact of autoradiolysis and allows for a more direct comparison of results to the microcosms.

2) Genetic analyses for crushed rock microcosms were limited to unamended bottles and lactate-amended bottles that exhibited reductive dechlorination beyond cDCE, not those with EVO and HRC amendment. Analysis of those treatments may provide additional information about the distribution of Dehalococcoides and the factors that limit reductive dechlorination beyond cDCE.

3) Pre-screening of the intact core samples may address concerns associated with heterogeneity by (a) selecting cores that encompass different features; (b) increasing the chance of observing activities such as reductive dechlorination of cDCE to VC and ethene based on preliminary DNA analysis; (c) shortening the
experimental timeline by selecting cores with a high potential for abiotic activity; and (d) avoiding unwanted abnormalities such as micro-fractures and impermeable regions.

4) Rock and groundwater samples should be well preserved in their original condition and used as soon as possible. Inappropriate sample handlings may impede the representativeness of subsequent microcosms. In this study, many precautions were taken, including: a) cores were cut under a nitrogen environment and sterile water was used to wet the saw; 2) core assembly was carried out under a continuous blanket of nitrogen to minimize air exposure; and 3) a cooling procedure was incorporated in the TIG welding process to avoid over-heating the cores. However, further improvements are possible, such as using clean and a more precise cutting tool to avoid metal deposits and reduce the variation in core lengths and shape. Since bacteria are sensitive to heat, a welding technique that generates less heat (e.g., laser welder) would mitigate this concern.

5) Additional characterization of the individual core samples is likely to improve data analysis and modeling. In this study, all cores were assumed to have the same porosity as the average sandstone porosity at the site (13%), whereas results indicated significantly lower porosity in pair #1. Tracer test and breakthrough curve analyses may provide information on rock porosity and help determine adsorption and retardation factors. End-of-incubation porosity measurements could also be helpful.
6) The comparison of rate estimates between intact rock core microcosms and crushed rock microcosms indicated potential inconsistencies in mineral surface areas. Mineralogy and surface area analyses would allow for a more fair comparison.

7) More treatments can be included in the design of experiments for intact core microcosms. For instance, it is worthwhile to have killed controls in addition to an unamended control, to exclusively evaluate abiotic transformation and better assess the contribution of different processes to the overall attenuation phenomena.

8) As discussed in Chapters 3 and 4, achieving initial groundwater saturation and compound distribution in the rock matrix is a major challenge for the intact core microcosms. Three additional cores were sacrificed at time zero to evaluate initial TCE distribution (not reported in Chapter 3). However, the method of core preservation was not ideal and caused significant TCE loss during transport and storage, so the results were no longer representative of the initial conditions. Mass balance checks and model simulations provided another way to confirm the initial assumption of uniform saturation; however, having direct measures is more compelling. One way to confirm saturation is comparing mass gain during pumping with core-specific porosity. A computed tomography (CT) scan with X-rays may be used to evaluate the distribution of groundwater inside the rock. Flushing the core with CO₂ prior to pumping may help achieve better core saturation as CO₂ will dissolve in water and be removed during pumping.
9) There is inevitably a small space between the Teflon wrapping around the cores and the stainless steel pipe into which they are inserted. The amount of space varies based on the thickness of the Teflon and the straightness of the cores, and is presumably occupied by air at the beginning of the experiment. Teflon is porous and TCE may absorb or diffuse through it; oxygen in the air can also diffuse through the Teflon and enter the rock and raise the redox level. The effects of this peripheral space have not been evaluated and should be taken into consideration in future designs.

10) Some minor deviations between the model and the experimental data may have been a consequence of not including dynamic absorption and desorption of VOCs, likely involving the rock and Teflon. Although these processes did not significantly impede the model applicability, they could be added in future versions to increase the modeling competence.

11) Rate estimation through modeling of changes in $\delta^{13}C$ was highly sensitive to variations in the enrichment factors. Values for enrichment factors cover a wide range, depending on the type of rock and structure of minerals. If $\delta^{13}C$ data is to be used for rate estimation, it is necessary to obtain site-specific enrichment factors. Another way to reduce the uncertainty in rate estimation is use of $^{14}C$-labeled compounds to prepare intact core microcosms. Fitting $\delta^{13}C$ data is an indirect means to predict the rate constant and therefore could be affected by other model parameter(s); monitoring production of $^{14}C$-labeled daughter products is a direct measurement and is likely to generate values that are conservative.
However, in a system with matrix diffusion, the model will still be an essential tool for interpreting the $^{14}\text{C}$ data. Since the weekly volume of sample available for $^{14}\text{C}$ measurements is small (e.g., 2 mL), protocols will have to be adapted for quantification of potentially low levels of $^{14}\text{C}$ in the samples.

12) Transformation processes other than reductive dechlorination appear to be significant and resulted in accumulation of $\text{CO}_2$ and NSR. However, the mechanisms of $\text{CO}_2$ and NSR production remain understudied and the components of NSR have not been fully identified. Acetic acid has been identified in NSR and it may form abiotically via hydrolysis of chloroacetylene (Fig. 1-1), yet this has not yet been experimentally verified. More research is needed to understand these abiotic pathways. Acetylene, another daughter product of abiotic transformation of chlorinated ethenes, is hard to detect in the field. Aerobic and anaerobic biodegradation of acetylene is known, yet the tools needed to document this process in situ are lacking. Specific assays should be developed to monitor these microbes and their unique enzymes, as indicators of in situ acetylene biodegradation.
6. APPENDICES
A. **Supplementary Material for Chapter 2**

A.1 **Sources of Samples**

This experiment required approximately 10 kg of crushed rock and 38 L of groundwater from the site. A corehole drilled in 2009 was selected as the location for rock samples. It was selected based on the following criteria:

1. An appropriate method of preservation was used during collection of rock samples. The cores were wrapped in mylar and then placed in a metalized vacuum bag. A nitrogen-purge vacuum system was used in the field to remove any air. The samples were shipped to the University of Guelph and kept refrigerated. These samples most closely represent the native state.

2. The corehole is located proximal to the corehole used in prior studies conducted by Darlington (137). Consequently, use of this corehole provided an opportunity to compare the consistency of the results.

3. The University of Guelph characterized the microbial composition of adjacent core samples (192).

Two nearby monitoring wells, one in the source zone and one down the plume well, were selected to provide a 50/50 (v/v) blend of groundwater. This mixture was deemed appropriate to represent the broad range of hydrogeochemical conditions anticipated across the site where enhanced biological reduction may be applied, based on the following reasons:

1. The source zone well has been the subject of special studies by Pierce (193) and Zimmerman (136), including analyzing groundwater samples collected from this
location for hydrochemistry and isotopes, dissolved gases, and compound specific isotope analysis (CSIA) of TCE and cDCE. Groundwater in this well also contains a reasonable composition of TCE and daughter products, including ethene and ethane, as documented in Zimmerman's work using the Snap Sampler®.

2. The plume well is believed to be located further down the flow path from the source and close to a well from which groundwater samples were obtained for the previous study by Darlington (3). This monitoring well was installed in August 2011 and compared to previous used well has a shorter screen length (1.52 m), therefore reducing uncertainty in the depth interval and conditions of the groundwater obtained from this location. Groundwater samples collected in September 2011 and analyzed for the concentration of VOCs showed a reasonable composition of TCE and daughter products (194). A set of high resolution hydrochemical and isotope data, including CSIA of TCE and cDCE were generated from groundwater samples set to be collected from this well as part of work directed by Amanda Pierce at the University of Guelph.
A.2 Mineral Salts Medium

Enrichment cultures were grown in an anaerobic mineral salts medium (MSM) (138), with the following constituents per liter of distilled deionized water: 10 mL of phosphate buffer (52.5 g of K$_2$HPO$_4$ per liter); 10 mL of salt solution (53.5 g of NH$_4$Cl per liter, 4.7 g CaCl$_2$·2H$_2$O per liter, 1.8 g of FeCl$_2$·H$_2$O per liter); 2 mL of trace mineral solution (0.3 g of H$_3$BO$_3$ per liter, 0.2 g of ZnSO$_4$·7H$_2$O per liter, 0.75 g of NiCl$_2$·6H$_2$O per liter, 1.0 g of MnCl$_2$·4H$_2$O per liter, 0.1 g of CuCl$_2$·2H$_2$O per liter, 1.5 g of CoCl$_2$·6H$_2$O per liter, 0.02 g of Na$_2$SeO$_3$ per liter, 0.1 g of Al$_2$(SO$_4$)$_3$·16H$_2$O per liter, 1 mL of concentrated HCl per liter); 2 mL of MgSO$_4$·7H$_2$O solution (62.5 g/L); 1 mL of resazurin solution (1.0 g/L); 50 mL of filter-sterilized NaHCO$_3$ solution (16.0 g/L); 10 mL of filter-sterilized yeast extract solution (5.0 g/L); 0.24 g of Na$_2$S·9H$_2$O and 0.1448 g of FeCl$_2$·H$_2$O.

The bicarbonate and yeast extract solutions were added after the medium was autoclaved and cooled. The container was then placed in an anaerobic chamber where the sulfide and ferric chloride were added. The medium was purged with 30%/70% CO$_2$/N$_2$ gas mix to reduce the pH; further adjustment to circumneutral was made via addition of 8 M NaOH solution or 6 M HCl.
### A.3 Preparation and Monitoring of Crushed Rock Microcosms

Crushed rock microcosms were prepared as described by Darlington et al. (129), with minor modifications. Treatments are listed in Table A-1.

**Table A-1.** Experimental design for crushed rock microcosm experiments; 12 replicates used for each treatment are shown.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Target TCE level</th>
<th>Target cDCE level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>without $^{14}$C</td>
<td>with $^{14}$C</td>
</tr>
<tr>
<td></td>
<td>0.62 µmol/bottle</td>
<td>0.62 µmol/bottle</td>
</tr>
<tr>
<td></td>
<td>(~1 mg/L)</td>
<td>(~0.5 µCi/bottle)</td>
</tr>
<tr>
<td>Unamended (UN)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Sulfate (S)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Lactate (L)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>lactate + sulfate (LS)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>EOS (E)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>EOS + sulfate (ES)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>HRC (H)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>HRC + sulfate (HS)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Water Control (WC)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Autoclaved control (AC)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>AC + sulfide (AS)</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>
Figure A-1 illustrate the preparation of microcosms. It should be noted that, in addition to the treatments shown in Table A-1, Figure A-1 also indicates the preparation of 18 bottles that were bioaugmented. The intent of these was to determine if the environmental conditions at the site are conducive to the growth of *Dehalococcoides*, rather than evaluating bioaugmentation as a remediation technology. Therefore the results were not shown in the main text but in this Appendix.

Crushed core samples were mixed together in a sterile plastic container in the anaerobic chamber. Each microcosm was prepared in the anaerobic chamber, by adding 20 g of crushed rock and 50 mL of groundwater (amended with 1 mg/L resazurin and added via a peristaltic pump) into a 160 mL serum bottle. Resazurin was added to serve as a redox indicator; a pink color at circumneutral pH is indicative of conditions above
approximately -110 mV; a clear color is indicative of a lower level. The bottles were capped with slotted grey butyl rubber septa and removed from the anaerobic chamber. The headspace of the bottles was then purged with high purity nitrogen for 1 min to remove hydrogen from the chamber’s atmosphere. They were recapped with Teflon-faced grey butyl rubber septa and crimp caps.

Autoclaved controls were constructed as described above (i.e., with crushed rock and site groundwater) and were then subjected to autoclaving at 121 °C for 60 min on three consecutive days. Autoclaved + sulfide controls were prepared the same way prior to adding a filter sterilized (0.1 µm) sulfide stock solution.

Water controls were prepared outside of the glove box, by adding 50 mL of distilled deionized water plus an equivalent volume of glass beads as the crushed rock into each serum bottle and capped without headspace purging.

To achieve the intended initial $^{14}$C level (~0.5 µCi/bottle) in the treatments with labeled compounds, a stock solution of $^{14}$C-TCE or $^{14}$C-cDCE prepared in groundwater was added. When necessary, TCE or cDCE saturated groundwater was added to achieve the intended initial TCE or cDCE concentration (~1 mg/L). To achieve similar concentrations in the treatments without labeled compounds, groundwater saturated solutions of TCE (60-70 µL/bottle) or cDCE (8-10 µL/bottle) were added. Stock solutions for addition of lactate, EVO, HRC, sulfide and sulfate were prepared as shown in Table A-2. For the bioaugmented microcosms, 0.5 mL of the MicroCED halorespiring culture (138, 195) was added along with lactate as the electron donor.
Table A-2. Electron donor, sulfate, and sulfide additions made to the microcosms.

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Na-lactate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EVO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>HRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C&lt;sub&gt;3&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;Na</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;36&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>C&lt;sub&gt;39&lt;/sub&gt;H&lt;sub&gt;56&lt;/sub&gt;O&lt;sub&gt;39&lt;/sub&gt;</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>112</td>
<td>284</td>
<td>956</td>
</tr>
<tr>
<td>Electron equivalent based on H&lt;sub&gt;2&lt;/sub&gt; (µeq/mmol)</td>
<td>4</td>
<td>36</td>
<td>52</td>
</tr>
<tr>
<td>Electron equivalent based on H&lt;sub&gt;2&lt;/sub&gt; (µeq/mg)</td>
<td>36</td>
<td>59</td>
<td>54</td>
</tr>
<tr>
<td>Electron equivalent based on fully oxidation (µeq/mmol)</td>
<td>12</td>
<td>108</td>
<td>150</td>
</tr>
<tr>
<td>Electron equivalent based on fully oxidation (µeq/mg)</td>
<td>107</td>
<td>177</td>
<td>157</td>
</tr>
</tbody>
</table>

**TCE Treatments**

<table>
<thead>
<tr>
<th></th>
<th>0.64 µmol/bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target TCE dose in the microcosm</td>
<td>0.64 µmol/bottle</td>
</tr>
<tr>
<td>Electron equivalent required for TCE reduction (6 eeq/mole)</td>
<td>3.86 µeq/bottle</td>
</tr>
<tr>
<td>Electron equivalent x 10 (safety factor)</td>
<td>38.6 µeq/bottle</td>
</tr>
<tr>
<td>Target e-donor dose in the microcosm (mg) &lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.07</td>
</tr>
<tr>
<td>Maximum electron equivalent provided by e-donor (µeq/bottle)</td>
<td>114.7</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; dose (mg/bottle) &lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;S·9H&lt;sub&gt;2&lt;/sub&gt;O dose (mg/bottle)</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**cDCE Treatments**

<table>
<thead>
<tr>
<th></th>
<th>0.64 µmol/bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target cDCE dose in the microcosm</td>
<td>0.64 µmol/bottle</td>
</tr>
<tr>
<td>Electron equivalent required for cDCE reduction (4 eeq/mole)</td>
<td>2.57 µeq/bottle</td>
</tr>
<tr>
<td>Electron equivalent x 10 (safety factor)</td>
<td>25.7 µeq/bottle</td>
</tr>
<tr>
<td>Target e-donor dose in the microcosm (mg) &lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.71</td>
</tr>
<tr>
<td>Maximum electron equivalent provided by e-donor (µeq/bottle)</td>
<td>76.4</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; dose (mg/bottle) &lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.4</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;S·9H&lt;sub&gt;2&lt;/sub&gt;O dose (mg/bottle)</td>
<td>2.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sodium lactate sample consisted of a 60% (m/m) syrup.

<sup>b</sup> Assuming the EVO consisted of 50% (by volume) oil (density = 0.92 g/mL) and 50% water.

<sup>c</sup> The target e-donor dose for TCE reduction was calculated based on expected H<sub>2</sub> production from each e-donor.

<sup>d</sup> Na<sub>2</sub>SO<sub>4</sub> dose calculated assuming complete oxidation of the e-donor and reduction of the sulfate to sulfide.
After preparation and initial monitoring, the bottles were returned to the anaerobic chamber for storage. They were removed periodically for headspace and liquid sampling.

The 12 microcosms that comprised each treatment were placed upside down in one plastic container (Fig. 2.5) and stored quiescently inside the glove box throughout the incubation period. Initially, electron donors were added monthly; the frequency of addition was gradually decreased to every three or four months. Sulfide addition started on a weekly basis and was gradually reduced to every three months. Sulfate additions were made every three to four months. VOCs were analyzed weekly at the beginning; over time, the frequency was reduced to bi-weekly, and then monthly or longer. $^{14}$C analysis was carried out every three to four months. $\delta^{13}$C analysis was carried out on three cDCE treatments without labeled compounds (UN, AC and WC) on days 0, 94, 186, 315 and 460.

The labeling system used to for the TCE microcosms was:

$$\text{TCE} - \text{Sx} - \text{y} - \text{Cz} - \#$$

where $x =$ group of triplicate bottles (I, II, III, or IV); $y =$ treatment (abbreviations in Table A-1); $z =$ y for yes, $^{14}$C-TCE was added, or n for no, $^{14}$C-TCE was not added; and $\#$ = bottle number within a group of triplicates (1, 2 or 3). The same labeling system was used for the cDCE microcosms, with cDCE replacing TCE.
A.4 VOCs, Anions, Organic Acids, and \textsuperscript{14}C Analysis

\textbf{VOCs}

VOCs were monitored by headspace analysis (140). PCE, TCE, cDCE, VC, ethene, ethane, acetylene, and methane were evaluated using a Hewlett-Packard 5890 Series II Gas Chromatograph, equipped with a flame ionization detector in conjunction with an 8 ft column packed with 1\% SP-1000 on 60/80 Carbopack-B (Supelco, Inc.). Nitrogen was used as the carrier gas. The temperature program was 60 °C for 2 min, ramp at 20 °C per min to 150 °C, ramp at 10 °C per min to 200 °C and hold for 1 min.

The gas chromatograph response to a headspace sample (0.5 mL) was calibrated to give the total mass of the compound (M) in that bottle (140). Assuming the headspace and aqueous phases are in equilibrium, the total mass present was converted to an aqueous-phase concentration with the following equation:

\[ C_l = \frac{M}{V_l + H_c V_g} \]  \hspace{1cm} (A-1)

where \( C_l \) is the concentration in the aqueous phase (\( \mu \)M), \( M \) is the total mass present (\( \mu \)mol/bottle), \( V_l \) is the volume of the liquid in the bottle (L), \( V_g \) is the volume of the headspace in the bottle (L), and \( H_c \) is the Henry’s law constant (gas concentration [mol/m\(^3\)]/aqueous concentration [mol/m\(^3\)].)
**Inorganic Ions**

Chloride, bromide, and sulfate were measured on a Dionex AS50 ion chromatography system, consisting of an autosampler (model #AS50), a pumping system (model #GP50 Gradient Pump) and a detector (model #CD25 Conductivity detector). Samples were prepared by filtration (0.20 µm PTFE, VWR) and injected through a 25 µL sample loop onto a Dionex guard column (AG9-HC, 4 mm x 50 mm), followed by an IonPac® AS9-HC anion-exchange column (4 mm x 250 mm). Eluant (9 mM Na₂CO₃) was delivered at 1.0 mL per min. Calibration standards ranged from 0.1 to 5.0 mM.

**Organic Acids**

Organic acids were analyzed on a Dionex Ultimate 3000 high performance liquid chromatography (HPLC) system composed of an autosampler (Ultimate 3000), a pumping system (Ultimate 3000), and a UV/Vis detector (Ultimate 3000 RS Variable Wavelength Detector) set at 210 nm. Samples were prepared by filtration (0.20 µm PTFE, VWR) and injected through a 100 µL sample loop onto an Aminex® HPX-87H ion exclusion column (300 mm x 7.8 mm; BioRad); 0.01 N H₂SO₄ served as the eluant, delivered at 0.6 mL per min. Standards for lactate, formate, acetate, propionate, and butyrate were used for calibration. Calibration standards ranged from 0.1 to 5.0 mM.
Stock solutions of $^{14}$C-TCE and $^{14}$C-cDCE were prepared by dissolving the neat compounds in site groundwater (50/50 mix). Prior to adding the stock solutions to the microcosms, they were cooled (4 °C) to maximize the amount partitioned to the aqueous phase. Approximately 85-110 μL of the $^{14}$C-TCE groundwater stock solution was added to the $^{14}$C-TCE microcosms to achieve an initial TCE amount of 0.58-0.68 μmol/bottle and an initial $^{14}$C level of 0.47-0.54 μCi/bottle. The groundwater from the site contained cDCE, which resulted in a trace level of unlabeled cDCE in the microcosms (0.058-0.074 μmol/bottle).

For the $^{14}$C-cDCE microcosms, approximately 8 μL cDCE saturated groundwater and 110-143 μL of the $^{14}$C-cDCE groundwater stock solution were injected to achieve an initial cDCE amount of 0.63-0.69 μmol/bottle and an initial $^{14}$C level of 0.46-0.52 μCi/bottle. Groundwater from the site contained TCE, which resulted in a trace level of unlabeled TCE (0.001-0.002 μmol/bottle).

Microcosms were equilibrated on an orbital shaker for 1 h prior to headspace analysis for VOCs. The solids were allowed to settle for at least another 0.5 h prior to liquid sampling. Samples were then removed for quantification of the total $^{14}$C in the bottles (100 μL liquid, 500 μL headspace).

$^{14}$C volatile compounds were separated by passing a 0.5 mL headspace sample through a CarboPack B column on the GC, the effluent from which was routed to a catalytic combustion tube where all the compounds were oxidized at 800 °C to CO$_2$ (Fig.
A-2). As different fractions eluted from the combustion tube, each was trapped in 3 mL 0.5 M NaOH, which was added to liquid scintillation cocktail for quantification of $^{14}$C.

Soluble $^{14}$C-products were measured by adding approximately 10 mL of slurry from a microcosm to a stripping chamber, which was then acidified (pH<4) with HCl (6 N) and sparged with N$_2$ to strip off the CO$_2$. Gas from the stripping chamber was passed into an absorption chamber containing 0.5 M NaOH (10 mL) (Fig. A-3). Liquid from both chambers (2 mL) was added to liquid scintillation cocktail and quantified for $^{14}$C. $^{14}$C remaining in the acidified chamber is referred to as non-strippable residue (NSR). This includes compounds that are non-volatile at a pH less than ~4, e.g., acetic acid, formic acid and glycolic acid (3), since it encompasses compounds that are non-volatile at low pH. Care was taken to sample only the liquid phase of the stripping chamber, to avoid any rock particles that might contain adsorbed $^{14}$C-TCE or $^{14}$C-cDCE. $^{14}$C remaining in the absorption chamber was presumptively considered to be $^{14}$CO$_2$. If levels were sufficiently high (i.e., >2 times the background level), confirmation of $^{14}$CO$_2$ was obtained by precipitation with barium hydroxide, as described in Darlington et al. (137).

$^{14}$C activity was counted on a PerkinElmer Tri-Carb® 2910 TR liquid scintillation analyzer. The distribution of $^{14}$C was calculated as the percentage of each $^{14}$C-compound or category in relation to the total initial $^{14}$C present in a microcosm. The compounds and categories included TCE, cDCE, VC, ethene, other VOCs (i.e., the VOCs that were detected by the GC-combustion method but were not attributable to a specific compound), $^{14}$C-NSR, $^{14}$CO$_2$, and unidentified compounds (i.e., $^{14}$C activity that was
detected via direct counts of the headspace and liquid but was not accounted for in the
procedures used to measure VOCs, CO$_2$, and NSR).

The procedures used to calculate the distribution of $^{14}$C are outlined in Darlington
et al. (137). This includes the percent distribution of each compound, the percentage of
$^{14}$C lost due to diffusion and adsorption during incubation, and the percentage of $^{14}$C in a
category referred to as “unaccounted for,” i.e., $^{14}$C that remained in a bottle that could not
be assigned to a specific VOC, NSR, or CO$_2$. 
**Figure A-2.** Procedure used for analysis of $^{14}$C volatile compounds.

**Figure A-3.** Procedure used for analysis of $^{14}$C-NSR and $^{14}$CO$_2$.  

- Headspace Sample  
  - CuO in tube furnace (800ºC)  
  - FID  
  - Gas Chromatograph  
  - 4-Port Valve  
  - 0.5 M NaOH in test tube, trapping $^{14}$CO$_2$  

- Fume hood  
  - N$_2$ gas  
  - 0.4 mL 6 N HCl  
  - Absorption chamber traps CO$_2$  
  - Stripping chamber traps NSR  
  - 10 mL sample  
  - 10 mL NaOH
A.5 qPCR and Sequencing

qPCR

*Dehalococcoides, bvcA, vcrA* and *tceA* were quantified using the TaqMan®-Probe-based qPCR method (Table A-2), adapted from Loffler and colleagues (141-142). Probes and primers were ordered from Integrated DNA Technologies, Inc. (IDT) as PrimeTime standard qPCR Assay. Standards were obtained from IDT as gBlocks® Gene Fragments. The composition of the reaction mixture is shown in Table A-3. Other supplies include the ABI MicroAmp® Fast Optical 96-Well Reaction Plate and MicroAmp® Optical Adhesive Film. The following temperature program was used on an Applied Biosystems® StepOnePlus™ Real-Time PCR System: 2 min at 50 °C, 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Detection limits for *Dehalococcoides, bvcA, vcrA* and *tceA* were 685-836 copies/mL, 1355-1652 copies/mL, 14000-17000 copies/mL and 156-190 copies/mL, respectively. A range was used for the detection limit due to the variation in sample volumes.

Primers reported in previous studies were used in qPCR for the following bacteria: *Dehalobacter* (144), *Desulfitobacterium* (144), *Sulfurospirillum* (196), *Desulfuromonas* (145), *Geobacter* (146) and *Geobacter lovleyi* (196) (Table A-2). SYBR® Green Dye-based qPCR was performed using a protocol adapted from previous studies (144). Primers and standards were obtained from IDT. The components in the qPCR master mix are shown in Table A-4. The following temperature program was used: 10 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 20 s at 56~61 °C (depending on the T_m of the primer pairs, as shown in Table A-2) and 30 s at 72 °C. A melting curve
from 50 °C to 99 °C was used at the end. Detection limits for *Geobacter* and *G. lovleyi* were 268-349 copies/mL and 134-190 copies/mL, respectively. Standard curves for *Dehalobacter*, *Desulfitobacterium*, *Sulfurospirillum* and *Desulfuromonas* were not prepared because preliminary qPCR analyses showed no amplification. Therefore, detection limits are not available for these bacteria.
Table A-2. Quantitative PCR primer sets, probes and annealing temperature (Tm).

<table>
<thead>
<tr>
<th>gene</th>
<th>primer and/or probe</th>
<th>Tm (°C)</th>
<th>Sequence (5’-3’, 5’-FAM-TAMRA-3’ for probes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehalococcoides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dhc 1200F</td>
<td></td>
<td>CTGGAGCTAATCCCCAAAGCT</td>
</tr>
<tr>
<td></td>
<td>Dhc 1271R</td>
<td>60</td>
<td>CAACTTCATGCAGGCGGG</td>
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<tr>
<td></td>
<td>Dhc 1240Probe</td>
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<td>TCCTCAGTTCGGAATTCGAGGTGCTGAA</td>
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<tr>
<td></td>
<td>bvcA 925F</td>
<td></td>
<td>AAAAGCAGCTTGCTATCAAGGAC</td>
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<tr>
<td>(D. sp. strain BAV1)</td>
<td>bvcA 1017R</td>
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<td></td>
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<tr>
<td></td>
<td>vcrA 1022F</td>
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<td>CGGGCGGATGACTATTTTT</td>
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<tr>
<td>(D. sp. strain GT)</td>
<td>vcrA 1093R</td>
<td>60</td>
<td>GAAATATCCGCTGCCCCCTCTC</td>
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<td>tceA 1270F</td>
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<td>(D. sp. strain FL2)</td>
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<td></td>
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<td>Desulfitobacterium</td>
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<td>GTACGACGAAGCGCTGCTG</td>
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<tr>
<td>spp.</td>
<td>Dsb 619R</td>
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<td>Desulfuromonas</td>
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<td>61</td>
<td>TGGAAACTGGGCAACTT</td>
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<tr>
<td>michiganensis</td>
<td>Dmich 852R</td>
<td></td>
<td>CTGATGCTGATTGCCTG</td>
</tr>
<tr>
<td>Sulfurospirillum</td>
<td>Sulfuro 114F</td>
<td>59</td>
<td>GCTAACCTGCCCTTAAGTG</td>
</tr>
<tr>
<td>halorespirans</td>
<td>Sulfuro 421R</td>
<td></td>
<td>GTTTACACACGGAAATGCT</td>
</tr>
<tr>
<td>Geobacter spp.</td>
<td>Geo 561F</td>
<td>56</td>
<td>GCGTGTAGGCCGTTTACTAA</td>
</tr>
<tr>
<td></td>
<td>Geo 825R</td>
<td></td>
<td>TACCCGCRACACCTAGTCT</td>
</tr>
<tr>
<td>Geobacter lovleyi</td>
<td>Geo 73F</td>
<td>59</td>
<td>CTTGCTCCTTCAATTAGTG</td>
</tr>
<tr>
<td></td>
<td>Geo 485R</td>
<td></td>
<td>AAAGAAACCGGGAATTAAC</td>
</tr>
</tbody>
</table>
### Table A-3. Components of a TaqMan® Probe-based qPCR reaction mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Solution (µM)</th>
<th>µL stock solution per 30 µL reaction</th>
<th>Final Concentration (nM)</th>
<th>Final amount per 30 µL reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2×</td>
<td>15</td>
<td>1×</td>
<td>-</td>
</tr>
<tr>
<td>Probe+Primers Mixture</td>
<td>6 (20×)</td>
<td>1.5</td>
<td>300</td>
<td>9 pmole</td>
</tr>
<tr>
<td>DNA template</td>
<td>-</td>
<td>TBD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>TBD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Power SYBR® Green Master Mix from Life Technologies.

<sup>b</sup> Volume was determined based on spectrophotometrically measured DNA concentration.

### Table A-4. Components of a SYBR® Green Dye-based qPCR reaction mix.

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Solution (µM)</th>
<th>µL stock solution per 25 µL reaction</th>
<th>Final Concentration (nM)</th>
<th>Final amount per 25 µL reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2×</td>
<td>12.5</td>
<td>1×</td>
<td>-</td>
</tr>
<tr>
<td>Forward Primer</td>
<td>6 (20×)</td>
<td>1.25</td>
<td>300</td>
<td>7.5 pmole</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>6 (20×)</td>
<td>1.25</td>
<td>300</td>
<td>7.5 pmole</td>
</tr>
<tr>
<td>DNA template</td>
<td>-</td>
<td>TBD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>TBD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> TaqMan® Gene Expression Master Mix was purchased from Life Technologies.

<sup>b</sup> Volume of DNA template and water was adjusted based on preliminary tests.
**Sequencing**

DNA samples were prepared according to the following protocol before being sent to the Clemson University Genomics Institute for sequencing analysis:

PCR was performed with the following components in each reaction: 18.5 µL water, 10 µL Q buffer, 6 µL MgCl₂ solution, 5 µL 10× buffer, 4 µL dNTPs, 1.25 µL forward primer (338F, 20 µM) appended with special overhang adapter sequences for Illumina sequencing (5’-TCGTCGCGAGCGTACGTGGTATAAGAGACAG-), 1.25 µL reverse primer (907R, 20 µM) with special overhang (5’-GTCTCAGCGGCTCGGAGATGTGTTATAAGAGACAG-), 1 µL 10× BSA, 0.5 µL Taq and 2.5 µL DNA template. The following temperature program was used: 10 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 1 min at 55 °C and 1 min at 72 °C. At the end, the temperature was held at 72 °C for 10 min and then reduced to 4 °C prior to further application.

DNA was checked via gel electrophoresis to verify the amplification. Purification was performed using the QIAquick® PCR Purification Kit following the manufacturer’s protocol. DNA was analyzed with a Qubit™ fluorometer or Nanodrop™ 2000 Spectrophotometer to determine the concentration and was normalized to the desired concentration for sequencing. The final product was sent to the Clemson University Genomics Institute or the Department of Biological Sciences for 16S Metagenomics sequencing.

DNA samples were sent to the Clemson Department of Biological Sciences for sequencing analysis directly after extraction.
A.6  **VOC Results for TCE Microcosms**

An overview of results is presented in Table A-5 with respect to the rate and extent of cDCE, VC and ethene formation.

**Table A-5.** Summary of the different degrees of reductive dechlorination observed in crushed rock microcosms. Numbers represent how many of the bottles within a treatment exhibited the indicated activity, out of 12 possible.

<table>
<thead>
<tr>
<th>TCE Treatment</th>
<th>WC</th>
<th>AC</th>
<th>AS</th>
<th>UN</th>
<th>S</th>
<th>L</th>
<th>LS</th>
<th>E</th>
<th>ES</th>
<th>H</th>
<th>HS</th>
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<tbody>
<tr>
<td>no reaction</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>TCE to cDCE, slow$^a$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>TCE to cDCE, fast$^a$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>7</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>cDCE to VC/ethene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TCE Treatment</th>
<th>WC</th>
<th>AC</th>
<th>AS</th>
<th>UN</th>
<th>S</th>
<th>L</th>
<th>LS</th>
<th>E</th>
<th>ES</th>
<th>H</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>no reaction</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TCE to cDCE, slow</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
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<tr>
<td>TCE to cDCE, fast</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>11</td>
<td>10</td>
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<td>12</td>
</tr>
<tr>
<td>cDCE to VC/ethene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TCE Treatment</th>
<th>WC</th>
<th>AC</th>
<th>AS</th>
<th>UN</th>
<th>S</th>
<th>L</th>
<th>LS</th>
<th>E</th>
<th>ES</th>
<th>H</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
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<td>12</td>
<td>12</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>cDCE to VC/ethene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>3</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TCE Treatment</th>
<th>WC</th>
<th>AC</th>
<th>AS</th>
<th>UN</th>
<th>S</th>
<th>L</th>
<th>LS</th>
<th>E</th>
<th>ES</th>
<th>H</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>no reaction</td>
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<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>12</td>
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<td>12</td>
</tr>
<tr>
<td>cDCE to VC/ethene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Slow = $< 0.055 \, \mu\text{M/d}$, fast = $> 0.055 \, \mu\text{M/d}$; rate based in the average amount of TCE reduced to cDCE divided by the time for the reaction to occur.
As a supplementary for Figure 2-1, Figure A-4 presents the average TCE results for each treatment over 500 days of incubation for the bottles with $^{14}\text{C}$-TCE added. In the sections that follow, representative results for each treatment are shown.

**Figure A-4.** Average concentration profile of TCE in different treatments with $^{14}\text{C}$-TCE added.
The extent and average rates of loss for TCE from the control treatments are summarized in Table A-6. These results pertain only to bottles in which reductive dechlorination was minor or not detectable. Bottles with $^{14}$C-TCE added were incubated for a similar length of time before the last batch was sacrificed (453-457 days). Bottles without $^{14}$C-TCE added were incubated up to approximately 2.7 times longer; nevertheless, the average rate of loss was no greater during the extended incubation period. Rates of loss were higher in the unamended, sulfate-amended, autoclaved controls and autoclaved treatments with sulfide added, likely due to adsorption and/or reaction with the crushed rock.

Most studies do not report results over such long incubation periods. For comparison purposes, the rates of loss for TCE reported in this study were lower than for other studies that employed water controls. For example, data from Ibbini (147) was used to calculate a loss rate of 0.50%/d for PCE in water controls over 100 days of incubation. Data from Johnston et al. (148) indicate loss rates of 0.14 to 0.21%/d for TCE in abiotic water over 306-395 days. Freedman and Gossett (149) reported a decrease in PCE from 618 to 228 nmol per bottle over 309 days of incubation, or an average loss rate of 0.20%/d. All of the percent rates of loss in this study were less than 0.14%/d; most were below 0.10%/d.
Table A-6. Summary of TCE loss in control treatments due to processes other than reductive dechlorination.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>14C added?</th>
<th>Maximum incubation period for microcosms with 14C-TCE</th>
<th>Maximum incubation period for microcosms without 14C-TCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time (d)</td>
<td>% Loss (ave ± stdev)</td>
</tr>
<tr>
<td>WC</td>
<td>Yes</td>
<td>457</td>
<td>28±3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>456</td>
<td>37±3</td>
</tr>
<tr>
<td>AC</td>
<td>Yes</td>
<td>453</td>
<td>52±2</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>455</td>
<td>62±9</td>
</tr>
<tr>
<td>AS</td>
<td>Yes</td>
<td>454</td>
<td>47±3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>457</td>
<td>49±6</td>
</tr>
<tr>
<td>UN</td>
<td>Yes</td>
<td>457</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>456</td>
<td>RD a</td>
</tr>
<tr>
<td>S</td>
<td>Yes</td>
<td>457</td>
<td>50±4</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>456</td>
<td>54±2</td>
</tr>
</tbody>
</table>

*RD represents “reductive dechlorination”; if reductive dechlorination of TCE was the predominant process in all bottles in a treatment, the rate of loss of TCE is not reported.*
Figure A-5 shows the results for TCE in one of the 12 water control bottles with 14C-TCE added and one of the 12 without 14C-TCE added. It is apparent that diffusive loss of TCE did occur with these extended incubation times. Figure A-6 provides representative VOC results for the autoclaved controls. All of the autoclaved controls experienced some degree of TCE loss. These losses were greater than the water controls, suggesting the occurrence of abiotic transformation and/or adsorption. In AC microcosms with 14C-TCE added, there was only a modest increase in 14CO2 and 14C-NSR above the water controls, so most of the loss observed based on head space measurements may be attributable to a higher degree of adsorption to crushed rock compared to glass beads.

Representative results for the autoclaved controls with sulfide added are shown in Figure A-7. The trends were similar to the autoclaved controls over a similar incubation period, indicating that addition of sulfide did not enhance abiotic degradation of TCE.

Figures A-8 and A-9 provide representative results for the unamended microcosms. Three types of behavior were observed. In 3 of the 12 microcosms without 14C-TCE added, there was comparatively rapid and complete reduction of TCE to cDCE in a time span of approximately two to four months; the cDCE formed appeared to persist for the remainder of the incubation period (Fig. A-8a). None of the 12 microcosms with 14C-TCE added exhibited this behavior. In all of the remaining microcosms without 14C-TCE added and 3 of the 12 microcosms with 14C-TCE added, there was a more gradual reduction of TCE to cDCE (Fig. A-8b, c). Reduction was more complete in the microcosms without 14C-TCE added, although most of the microcosms with 14C-TCE added were incubated for less time, as triplicates were sacrificed to determine the
distribution of \(^{14}\)C. In the remaining microcosms with \(^{14}\)C-TCE added, there was no discernible reductive dechlorination of TCE to cDCE (Fig. A-9).

Representative results for the microcosms amended with only sulfate are shown in Figures A-10 and A-11. The same trends described above were observed, i.e., there was rapid reduction of TCE to cDCE in 8 of the 24 total microcosms (Fig. A-10a), more gradual reduction in 7 of 24 (Fig. A-10b, c), and no apparent reduction in 9 of 24 (Fig. A-11).

Taken together, results from the unamended and the sulfate amended microcosms suggest that the potential for reductive dechlorination of TCE to cDCE exists, but is limited mainly by the lack of an available electron donor and possibly the heterogeneous distribution of chlororespiring microbes. In microcosms that did exhibit reduction of TCE to cDCE, no other volatiles accumulated to a significant extent, including VC, ethene, or ethane.

Addition of lactate had a substantial impact. In 23 out of the 24 lactate-amended microcosms, TCE was reductively dechlorinated to cDCE; representative results are given in Figure A-12 for bottles that did and did not receive \(^{14}\)C-TCE. This result is consistent with the widespread occurrence of cDCE in situ, and the results with the unamended microcosms. Less expected, however, was the further reduction of cDCE to VC and ethene, since these compounds are detected in situ at only low levels. TCE reduction to VC and ethene occurred in all 12 lactate-amended bottles without \(^{14}\)C-TCE added and 3 of the 12 bottles with \(^{14}\)C-TCE added. In the remaining 9 bottles with \(^{14}\)C-TCE added, all displayed rapid reduction of TCE to cDCE, except for 1 bottle that
exhibited no discernible reductive dechlorination. Reduction of cDCE to VC occurred at a rate comparable to that for reduction of TCE to cDCE; however, subsequent reduction of VC to ethene occurred at a much slower rate. The slower rate of VC reduction to ethene was presumably not a consequence of a lack of electron donor, since repeated additions of lactate were made over time, in considerable excess of the electron equivalents needed to complete the reduction. In a number of the microcosms, methane started to accumulate after several years of incubation, indicative of an abundance of electron donor.

Amendment with sulfate in addition to lactate had a competitive effect on the electron donor. In all 12 lactate + sulfate-amended microcosms that did not receive $^{14}$C-TCE, TCE was reductively dechlorinated to cDCE, but cDCE was further reduced to VC and ethene in only two bottles. Out of the 12 lactate + sulfate-amended microcosms that received $^{14}$C-TCE, 10 bottles exhibited a high rate of TCE reduction to cDCE; among these, three exhibited further reduction of cDCE to VC and ethene. In the other two bottles with $^{14}$C-TCE added, one displayed a slow rate of TCE reduction to cDCE and one showed no discernible reductive dechlorination.

Figure A-13 presents representative VOC results for one of the lactate + sulfate-amended microcosms that completely reduced TCE to VC, and partially to ethene, in bottles that did and did not receive $^{14}$C-TCE. Figure A-14 shows the VOC results for one of the lactate + sulfate-amended TCE microcosms that reduced TCE to cDCE but no further (with and without $^{14}$C-TCE). Figure A-15 provides representative results for the
microcosms that showed slow (Fig. A-15a) or no discernible reductive dechlorination (Fig. A-15b).

Figure A-16 presents representative VOC results for the EVO-amended treatment in which TCE was reduced to cDCE, with and without $^{14}$C-TCE added. Eleven of the 12 EVO-amended TCE microcosms exhibited rapid reduction of TCE to cDCE; in one of the bottles with $^{14}$C-TCE added, reduction stalled after TCE had dropped to a low level.

Figure A-17 presents representative VOC results for EVO + sulfate-amended microcosms that reduced TCE to cDCE, with and without $^{14}$C-TCE added. The pattern in reductive dechlorination was nearly identical to the treatment with only EVO added. Representative results for one of the bottles in which reduction of TCE to cDCE did not occur are shown in Figure A-18.

Similar to EVO, most of the 24 HRC-amended TCE microcosms exhibited reduction of TCE to cDCE, but no further. Figure A-19 presents representative VOC results for microcosms that performed this reduction at a high rate, with and without $^{14}$C-TCE. Figure A-20 shows VOC results for an HRC-amended TCE microcosm that did not reduce TCE or did so at a relatively slower rate.

Figure A-21 presents representative VOC results for an HRC + sulfate-amended microcosm in which TCE was reduced to cDCE, with and without $^{14}$C-TCE added. A high rate of reduction occurred in all 24 microcosms.
Figure A-5. TCE results for one of the 12 water control bottles (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Figure A-6. Representative VOC results for autoclaved controls (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Figure A-7. Representative VOC results for autoclaved controls + sulfide amendment (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Figure A-8. Representative VOC results for unamended microcosms (a, b) without $^{14}$C- TCE added and (c) with $^{14}$C- TCE added.
Figure A-9 Representative VOC results for unamended microcosms with $^{14}$C-TCE added. A modified figure of panel (b) is shown in Figure 2-1b.
Figure A-10. Representative VOC results for sulfate amended microcosms that reduced TCE to cDCE (a, b) without $^{14}$C-TCE added and (c) with $^{14}$C-TCE added. A modified figure of panel (b) is shown in Figure 2-1c.
Figure A-11. Representative VOC results for sulfate amended microcosms (a) without \textsuperscript{14}C-TCE added and (b) with \textsuperscript{14}C-TCE added that did not reduce TCE to cDCE.
Figure A-12. Representative VOC results for lactate amended microcosms (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added. A modified figure of panel (a) is shown in Figure 2-1e.
Figure A-13. Representative VOC results for lactate + sulfate amended microcosms that reduced TCE to VC and ethene (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Figure A-14. Representative VOC results for lactate + sulfate amended microcosms (a) without $^{14}$C-TCE added and (b) with $^{13}$C-TCE added that reduced TCE to cDCE.
Figure A-15. Representative VOC results for lactate + sulfate amended microcosms with $^{14}$C-TCE added that showed (a) partial or (b) no reductive dechlorination of TCE to cDCE.
Figure A-16. Representative VOC results for EVO amended microcosms (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added. A modified figure of panel (a) is shown in Figure 2-1d.
Figure A-17. Representative VOC results for EVO + sulfate amended microcosms that reduced TCE to cDCE (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Figure A-18. Representative VOC results for EVO + sulfate amended microcosms with $^{14}$C-TCE added that did not reduce TCE to cDCE.
Figure A-19. Representative VOC results for HRC amended microcosms that reduced TCE to cDCE (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Figure A-20. Representative VOC results for HRC amended microcosms with $^{14}$C-TCE added that (a) did not reduce TCE to cDCE or (b) partially reduce TCE to cDCE.
Figure A-21. Representative VOC results for HRC + sulfate amended microcosms (a) without $^{14}$C-TCE added and (b) with $^{14}$C-TCE added.
Bioaugmentation

Additional microcosms were subjected to bioaugmentation to determine if something in the rock or groundwater at this site might be inhibitory to the activity of *Dehalococcoides*, which are required for complete dechlorination of TCE to ethene. Figure A-22 provides representative VOC results for different doses with the MicroCED halorespiring culture. Figure A-23 focuses on the rate of ethene production, which was proportional to the inoculum dose. These results confirmed that rock and groundwater are compatible with complete dechlorination. As these results were obtained, it became apparent that there are indigenous *Dehalococcoides* present, since reduction of cDCE to VC and ethene occurred in many of the lactate-amended microcosms.
Figure A-22. Representative VOC results for bioaugmented TCE microcosms at (a) 1%, (b) 0.01% and (c) 0.001% inoculum levels (v/v). The microcosms with 1% inoculum were set up with \(^{14}\text{C}\)-TCE added; the microcosms with the other two inoculum levels (0.01% and 0.001%) were set up without \(^{14}\text{C}\)-TCE added.
Figure A-23. Comparison of ethene production in microcosms at different levels of bioaugmentation.
A.7 VOS Results for cDCE Microcosms

An overview of cDCE results is presented in Table A-5 with respect to the rate and extent of VC and ethene formation.

As a supplementary for Figure 2-2, Figure A-24 presents the average cDCE results for each treatment over 500 days of incubation for the bottles with $^{14}$C-cDCE added. In the sections that follow, representative results for each treatment are shown.

Figure A-24. Average concentration profile of TCE in different treatments with $^{14}$C-TCE added.
The extent and average rates of loss for cDCE from the control treatments are summarized in Table A-7. Bottles with $^{14}$C-cDCE added were incubated for a similar length of time before the last one was sacrificed (455-460 days). Bottles without $^{14}$C-cDCE added were incubated up to approximately 2.4 times longer; nevertheless, the average rate of loss was similar or smaller during the extended incubation period. Rates of loss were higher in the unamended, sulfate-amended, autoclaved controls and autoclaved treatments with sulfide added, likely due to adsorption and/or reaction with the crushed rock. The rates of loss for cDCE reported in this study were lower than those for PCE and TCE in other studies that employed water controls (147-149). All of the percent rates of loss in this study were less than 0.09%/d; some were as low as 0.02%/d. Losses for cDCE were lower than for TCE, perhaps due to a slower rate of cDCE diffusion through holes in the septa, a greater extent of TCE adsorption to surfaces, and in bottles with $^{14}$C added, a greater extent of autoradiolysis for TCE.
Table A-7. Summary of cDCE loss in control treatments.

<table>
<thead>
<tr>
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<th>(^{14}\text{C} ) added?</th>
<th>Time (d)</th>
<th>% Loss (ave ± stdev)</th>
<th>Rate of loss (%/d)</th>
<th># of data points</th>
<th>Time (d)</th>
<th>% Loss (ave ± stdev)</th>
<th>Rate of loss (%/d)</th>
<th># of data points</th>
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<td>459</td>
<td>7±8</td>
<td>0.015</td>
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<td>10±5</td>
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<td>12</td>
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<td>33±5</td>
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<td>0.063</td>
<td>12</td>
<td>1091</td>
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<td>No</td>
<td>456</td>
<td>34±4</td>
<td>0.075</td>
<td>12</td>
<td>894</td>
<td>39±5</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure A-25 shows the results for cDCE in one of the 12 water control bottles with \(^{14}\)C-cDCE added and one of the 12 without \(^{14}\)C-cDCE added. The extent of diffusive loss of cDCE was minor when considering the extended incubation times.

Figure A-26 provides representative VOC results for the cDCE autoclaved controls. All of the autoclaved controls experienced some degree of cDCE loss. These losses were greater than the water controls, suggesting that most of the loss of cDCE was due to adsorption.

Representative results for the autoclaved controls with sulfide added are shown in Figure A-27. The trends were similar to the autoclaved controls over a similar incubation period, indicating that addition of sulfide did not enhance abiotic degradation of cDCE.

Figure A-28 provides representative results for the unamended microcosms. Unlike the unamended microcosms that received TCE, in the 24 that received cDCE there was no evidence of reductive dechlorination. The rate and extent of decrease in cDCE was similar to what was observed in the autoclaved controls.

Representative VOC results for the microcosms amended with only sulfate are shown in Figure A-29. Taken together, results from the unamended and the sulfate amended microcosms are consistent with field data that indicate a lack of significant reductive dechlorination of cDCE or sulfate reduction.

Addition of lactate had a substantial impact. In all 12 of the microcosms to which lactate was added without \(^{14}\)C-cDCE, cDCE was fully dechlorinated to VC, which was then dechlorinated to ethene at a slower rate. The same trend was observed in the TCE microcosms amended with lactate. In 4 of the 12 bottles that received \(^{14}\)C-cDCE,
reductive dechlorination of cDCE to VC and ethene was also observed. These microcosms were not incubated as long as the ones that did not receive $^{14}$C-cDCE, which likely explains the lower frequency of observing significant levels of reductive dechlorination. The same trend was observed in the $^{14}$C-TCE microcosms amended with lactate. Representative results for the VOCs are shown in Figure A-30 for microcosms that exhibited complete dechlorination and in Figure A-31 for two of the microcosms that received $^{14}$C-cDCE and did not exhibit significant dechlorination. In a number of the microcosms, methane started to accumulate after several hundred days of incubation (e.g., Fig. A-30b), indicative of an abundance of electron donor.

The occurrence of cDCE and VC reduction in amended treatments provided further evidence that conditions at the site are deficient in electron donor; in locations where donor has been available, it has supported reduction of TCE to cDCE. The microcosm results confirm that if sufficient donor became available, further reduction is possible in at least some locations due to the presence of indigenous *Dehalococcoides*.

As in the lactate-amended TCE microcosms, complete sulfate consumption occurred in all of the lactate-amended bottles that received cDCE, suggesting that the addition of lactate lowered the redox level and stimulated indigenous sulfate reducing bacteria (Fig. A-39).

Amendment with sulfate in addition to lactate had a competitive effect on the consumption of electron donor. Reduction of cDCE to VC and ethene occurred in only 3 of the 12 bottles with lactate and sulfate added, compared to all 12 without sulfate added. Representative results for two of the bottles that exhibited reductive dechlorination (both
without $^{14}$C-cDCE added) are shown in Figure A-32; representative results for bottles that did not exhibit any reductive dechlorination are shown in Figure A-33.

Figures A-34 and A-35 present representative VOC results for the EVO-amended and the EVO + sulfate-amended treatments, respectively. Unlike lactate, EVO did not promote reductive dechlorination of cDCE to VC and ethene, even though it was effective in promoting reduction of TCE to cDCE.

Results for the HRC-amended and HRC + sulfate-amended microcosms that received cDCE were very similar to those for EVO and EVO + sulfate. Figures A-36 and A-37 present representative VOC results, which indicate that HRC did not stimulate reductive dechlorination of cDCE to VC and ethene.
Figure A-25. Representative cDCE results for water controls (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-26. Representative VOC results for autoclaved controls (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-27. Representative VOC results for autoclaved controls + sulfide amendment (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-28. Representative VOC results for unamended microcosms (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added. A modified figure of panel (a) is shown in Figure 2-2b.
Figure A-29. Representative VOC results for sulfate amended microcosms (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-30. Representative VOC results for lactate amended microcosms (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added. A modified figure of panel (a) is shown in Figure 2-2c.
Figure A-31. Representative VOC results for lactate amended microcosms with $^{14}$C-cDCE added that did not show discernible reductive dechlorination of cDCE.
Figure A-32. Representative VOC results for lactate + sulfate amended microcosms without $^{14}$C-cDCE added that produced VC and ethene.
Figure A-33. Representative VOC results for lactate + sulfate amended microcosms that did not reduce cDCE to VC or ethene (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-34. Representative VOC results for EVO amended microcosms (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-35. Representative VOC results for EVO + sulfate amended microcosms (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-36. Representative VOC results for HRC amended microcosms (a) without $^{14}$C-cDCE added and (b) with $^{14}$C-cDCE added.
Figure A-37. Representative VOC results for HRC + sulfate amended microcosms (a) without 14C-cDCE added and (b) with 14C-cDCE added.
A.8  Acetylene Production

The amounts of acetylene in microcosms with $^{14}$C-TCE or $^{14}$C-cDCE added are shown in Figures A-38.

![Acetylene production in microcosms](image)

**Figure A-38.** Acetylene production in (a) TCE microcosms with $^{14}$C-TCE added and (b) cDCE microcosms with $^{14}$C-cDCE added. Percentage recovery was calculated assuming an initial TCE or cDCE level of 0.6 µmol/bottle.
A.9  Sulfate Consumption

Sulfate consumptions in microcosms with $^{14}$C-TCE or $^{14}$C-cDCE added are shown in Figures A-39.

Figure A-39. Sulfate results for (a) TCE microcosms with $^{14}$C-TCE added, and (b) cDCE microcosms with $^{14}$C-cDCE added. Each bar represents the average for triplicate bottles and duplicate samples per bottle; error bars represent standard deviations.
Complete $^{14}$C distributions in microcosms with $^{14}$C-TCE or $^{14}$C-DCE added are shown in Figures A-40.

**Figure A-40.** Recovery of $^{14}$C-compounds in (a) $^{14}$C-TCE microcosms and (b) $^{14}$C-cDCE microcosms. Percentages were calculated based on the initial $^{14}$C-TCE or $^{14}$C-cDCE level in the microcosms. Each bar represents the average of triplicate bottles; error bars represent the standard deviation. Determination of a first order transformation rate for (c) TCE and (d) cDCE based on accumulation of $^{14}$CO$_2 + ^{14}$C-NSR in the unamended microcosms. Numbers on the x-axis indicate sampling time: 1=90 day; 2=180 day; 3=320 (TCE) or 315 day (DCE); 4=460 day.


A.11 cDCE Enrichment Factor Calculation

Enrichment factors ($\varepsilon$) for the unamended and autoclaved control treatments were determined by plotting the $\delta^{13}$C data (y-axis) versus the natural logarithm of the ratio of cDCE remaining ($C$) to the initial cDCE ($C_o$) (x-axis). The x-axis was determined based on accumulation of $^{14}$C products (i.e., CO$_2$ + NSR), as follows:

$$\frac{C}{C_o} = \frac{^{14}C-cDCE_o - (^{14}C-VC) - (^{14}CO_2) - (^{14}C-NSR) - (^{14}C-other VOCs)}{^{14}C-cDCE_o}$$  \hspace{1cm} (A-2)

where $^{14}C-cDCE_o$ represents the total cDCE per bottle at time zero (based on liquid and headspace counts); the other products were determined at four time points. Using this approach, the enrichment factor was -75 ± 24‰ (95% confidence interval) for unamended live microcosms and -30 ± 9‰ for autoclaved controls. This magnitude of $\delta^{13}$C enrichment for cDCE is higher than other studies in which Fe$^0$ and MnO$_4^-$ were evaluated (64-67) (Table 1-1). However, no previous studies were found that evaluated $\delta^{13}$C enrichment of cDCE with minerals encountered in the site sandstone/siltstone, including iron sulfides, pyrite, fougerite, magnetite, biotite, vermiculite, and quartz (63). Furthermore, no previous studies have attempted to measure fractionation for an anaerobic pathway based on formation of CO$_2$ + NSR.
A.12 Enrichment Cultures

Enrichment cultures were prepared with PCE, TCE, cDCE, or VC as the electron acceptor. Representative results for one of the bottles that received cDCE or TCE are shown in Figure A-41; results for the duplicate bottles were similar. TCE and cDCE were repeatedly dechlorinated at a high rate, with accumulation of VC, which then underwent a slower rate of dechlorination to ethene. In contrast, the enrichment that received only VC did not exhibit any reductive dechlorination (Fig. A-42b). The first three additions of PCE were reduced at a high rate to cDCE, but there was no activity on the fourth addition (Fig. A-42a). In an attempt to restart activity in these bottles, TCE was added on day 37, but that had no impact. These results suggest that the enrichment developed from the site microcosms contain microbes capable of respiring TCE and cDCE, but not PCE or VC. The activity on PCE and VC appears to be cometabolic.
Figure A-41. VOC results for enrichment cultures that respired (a) cDCE and (b) TCE.
Figure A-42. VOC results for enrichment cultures provided with (a) PCE and (b) VC.
**A.13 Sequencing and qPCR**

To help ascertain which types of microbes are responsible for the dechlorination activity observed in the microcosms, DNA was extracted and subjected to Illumina 16S rRNA gene sequencing. Only DNA extracted from the aqueous phase samples were used for this purpose. *Dehalococcoides* spp., *Dehalococcoides mccartyi*, and *Geobacter lovleyi* were detected only in microcosms that exhibited TCE and cDCE reduction to ethene, while *Geobacter* were detected in all of the microcosms tested (Fig. A-43). It should be noted that the reliability of Illumina sequencing results diminishes below the genus level, so further characterization is needed to improve the level of confidence in the results at the species level, i.e., the ability to distinguish *Dehalococcoides mccartyi* from other *Dehalococcoides* spp., and *Geobacter lovleyi* from other *Geobacter* spp.

Lima et al. (52) also evaluated site samples for the presence of *Dehalococcoides* but did not detect any. This is likely a consequence of the in situ samples being deficient in electron donor, i.e., a low level of *Dehalococcoides* are likely present but their numbers are sufficiently low to avoid detection. Only after enhancing their growth in the microcosms by addition of lactate did their in situ presence become apparent.

The only *Dehalococcoides* species identified was *mccartyi* (Fig. A-43). This is notable since *D. mccartyi* is unable to respire VC to ethene; instead, the transformation is cometabolic and occurs at a noticeably lower rate, as was observed in the microcosms and the enrichments. It is also consistent with the observation that the enrichments were unable to transform VC when it was the only chlorinated ethene provided (Fig. A-42b).
Additional research is needed to better characterize the *Dehalococcoides* at this site, since the Illumina sequencing results are not sufficiently reliable below the genus level.

The absence of *Dehalococcoides* in the microcosms that reduced TCE only to cDCE suggests that this first dechlorination step was mediated by a different type of microbe, e.g., *Geobacter*. The ubiquity of *Geobacter* in the samples that were evaluated is consistent with their metabolic versatility and their ability to use ferric iron as an electron acceptor with a variety of electron donors. *Geobacter lovleyi* is well known for its ability to reduce PCE and TCE to cDCE. However, *G. lovleyi* was not detected in the unamended samples that exhibited TCE reduction to cDCE. This implies that this site has a species of *Geobacter* that has not previously been shown to perform chlororespiration. Addition of lactate may have given *G. lovleyi* a kinetic advantage that favored their growth over that of other TCE respiring *Geobacter*.

Illumina 16S rRNA gene sequencing results for *Geobacter* and *Geobacter lovleyi* genes and *Dehalococcoides* and *Dehalococcoides mccartyi* genes in the enrichment cultures was in progress at the time this report was submitted. The final results will be communicated in an addendum to this report as soon as they are available.

While Illumina 16S rRNA gene sequencing provides a qualitative indication of the types of microbes present, qPCR provides quantitative information.

Figure A-44a shows the qPCR results for *Dehalococcoides* and three organohalide respiring genes (*tceA*, *vcrA*, and *bvcA*) for DNA extracted from the rock. The results are consistent with the groundwater results, that only *Dehalococcoides* and *tceA* amplified in DNA extracted from the crushed rock microcosms that were amended
with lactate and exhibited significant ethene formation, with the exception of a low level of \textit{bvcA} in one of the cDCE amended bottles. Figure A-44b shows the qPCR results from the enrichment cultures, and the detection of \textit{Dehalococcoides} and \textit{tceA} was consistent with the results of microcosms. Due to active reductive dechlorination, \textit{Dehalococcoides} and \textit{tceA} levels were an order of magnitude higher in the TCE and cDCE enrichments compared to the PCE and VC enrichments, and lower concentrations in the PCE and VC treatments were likely a consequence of carry-over from the inoculum. A low level of \textit{vcrA} was detected in the bottles that received TCE and cDCE, suggesting that it may have played a minor role in respiration of TCE and cDCE compared to \textit{tceA}.

As in the microcosms, \textit{Geobacter} were ubiquitous in the enrichments (Fig. A-45). Levels were highest in the TCE enrichment, providing additional support to the hypothesis that \textit{Geobacter} were responsible for dechlorination of TCE to cDCE. The higher levels in the PCE enrichment compared to cDCE and VC may be reflect a longer period of survival of the microbes as PCE was undergoing cometabolic reduction to cDCE. The presence of \textit{G. lovleyi} in all of the enrichment cultures is consistent with the previous observation that it was only detected in lactate-amended microcosms, since all of the enrichment cultures received lactate.

Table A-8 summarizes the type of activities in terms of chlororespiration and cometabolic reduction in the unamended, lactate-amended, EVO-amended and HRC-amended microcosms. Although sequencing and qPCR were not performed with the EVO-amended and HRC-amended microcosms, reduction of TCE to cDCE in these treatments is presumed to be metabolic based on the similar rates and extents of activity.
in comparison to the lactate-amended microcosms. Table A-9 summaries the different types of dechlorinators and dehalogenase enzymes most likely responsible for reductive dechlorination of TCE, cDCE and VC in the microcosms, based on the sequencing and qPCR results. As highlighted by red circles and arrows, *Geobacter* were most likely responsible for reductive dechlorination of TCE to cDCE and *Dehalococcoides mccartyi* were most likely responsible for reduction of cDCE to VC and VC to ethene.

Table A-10 summarizes the sequence results for *Pelobacter acetylenicus*. 
Figure A-43. Percentages of (a) *Dehalococcoides* and *D. mccartyi* genes and (b) *Geobacter* and *G. lovleyi* genes determined from Illumina 16S rRNA gene sequencing of samples from crushed rock microcosms and enrichment cultures (indicated by cross-hatching). DNA was extracted from groundwater in the microcosms and analyzed via the Clemson University Genomics Institute (no cross-hatching) or courtesy of B. J. Campbell, Clemson University, Dept. Biological Sciences (indicated by an asterisk).
Figure A-44. Representative qPCR results for the *Dehalococcoides* 16S rRNA gene and reductive dehalogenase gene *tceA* (*verA* and *bvcA* were below the detection limits so no data are shown) in (a) rock samples from the microcosms and (b) enrichment culture. For each microcosm treatment, 2 out of 12 bottles were analyzed, which are referred to as 1 and 2 in the legend; error bars represent standard deviations. An asterisk after the treatment name indicates that all tested genes in the samples were below the limits of detection.
Figure A-45. Representative qPCR results for (a) the *Geobacter* 16s rRNA gene and (b) the *Geobacter lovleyi* gene, in enrichment cultures fed with PCE, TCE, DCE and VC. Each bar is the average of 4 measurements from a single bottle; error bars represent standard deviations.
Table A-8. Summary of metabolic and co-metabolic activities in selected treatments.

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<th>Compound</th>
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<th>DCE→VC</th>
<th>VC→ethene</th>
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<td>Unamended, EVO, HRC</td>
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<tr>
<td></td>
<td>Lactate</td>
<td>M</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>cDCE</td>
<td>Unamended, EVO, HRC</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>M</td>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

Table A-9. Summary of dechlorinators and enzymes responsible for reductive dechlorination in microcosms and enrichment cultures.

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<th>cDCE</th>
<th>VC</th>
<th>ethene</th>
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<tr>
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<td>tceA</td>
<td>tceA</td>
<td>vcrA</td>
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<tr>
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<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Dehalococcoides mccartyi strain 195; Dehalococcoides sp. strain FL2</td>
<td>M</td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
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<td>--</td>
<td>--</td>
<td>M</td>
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<tr>
<td>Dehalococcoides. sp. BAV1</td>
<td>--</td>
<td>--</td>
<td>M</td>
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-234-
Table A-10. Summary of detection of *Pelobacter acetylenicus* in sequencing analysis.

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<td></td>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Pelobacter acetylenicus</em> count</td>
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<td>82</td>
<td>46</td>
<td>193</td>
</tr>
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<td></td>
<td></td>
<td>542</td>
</tr>
<tr>
<td>% out of total genus identified</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
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B. Supplementary Material for Chapter 3

B.1. Intact Core Microcosms Setup and Monitoring

A conceptual model for the intact core microcosms is illustrated in Figure B-1.

![Conceptual model for the intact core microcosms](image)

Figure B-1. Conceptual model for the intact core microcosms.

The step-by-step procedure used to assemble the intact core microcosms is shown in Figure B-2. Each 3 inch long core was sandwiched between two 1 inch stainless steel end caps and (Fig. B-2a) surrounded by a layer of Teflon tape and then a heat-shrinkable Teflon® sleeve (Fig. B-2b and c). The design of the end caps is shown in Figure B-3. Self-adhesive red rubber tape and hose clamps were wrapped around the cores to provide the confining pressure to prevent peripheral flow during pumping of groundwater through the cores (Fig. B-2d and e). The rock cores were then saturated with groundwater amended with TCE, bromide and resazurin. The purpose of passing groundwater through the cores was to replace TCE lost during core sampling and processing; to increase the
initial TCE concentration to a level that improved the likelihood of observing activity within the timeframe of the project; to add bromide as a conservative tracer; and to add resazurin as a redox indicator. To confirm the concentration of TCE in the cores, triplicate intact core microcosms were sent to the University of Guelph, where the cores were sacrificed for analysis (courtesy of Dr. Beth Parker; a copy of the complete results is available upon request). The resulting TCE concentration in the pore groundwater was approximately 6.7-19.5 mg/L. Preliminary modeling indicated this concentration range resulted in sufficient diffusion into the top chamber such that the TCE level in that chamber was several milligrams per liter of TCE, which was readily quantifiable.

Groundwater was pumped through the rock cores using a pump and a bladder accumulator (Fig. B-2f); the bladder was a closed system and therefore allowed for transfer of groundwater without volatilization losses of TCE. Figure B-4 illustrates how the groundwater was prepared and transferred to the bladder accumulator. The groundwater (amended with bromide and resazurin) was poured into a 2 L aspirator bottle that was attached to a vacuum, to remove dissolved gasses (Fig. B-4a). The groundwater was then allowed to flow by gravity into a Tedlar bag; the headspace of the aspirator bottle was sparged with high purity N2 to prevent the groundwater from coming in contact with air (Fig. B-4b). TCE saturated groundwater was injected through a septum in the Tedlar bag to achieve the desired concentration (Fig. B-4c). The Tedlar bag was then connected to the lower part of the bladder (Fig. B-4d) and tap water in the upper half of the bladder was drawn out, in order to pull groundwater from the Tedlar bag into the lower part of the bladder (Fig. B-4e).
Groundwater was then pumped from the bladder into the top chamber through one of the two holes, with the other hole vented to the atmosphere. Once the chamber was filled, the other hole was sealed, the microcosm was inverted (top cap at the bottom), and tubing was connected to the single hole in the bottom cap that discharged to a glass bottle that collected the excess water (Fig. B-2f). Groundwater was pumped through the core at a pressure of approximately 35 pounds per square inch, entering from the top cap and exiting through the bottom cap. Pumping was stopped once the concentration of bromide in the effluent equaled that in the bladder. The holes on the end caps were then sealed with threaded stainless steel plugs (Fig. B-2g).

Evidence to indicate that the cores were saturated once pumping was terminated includes mass balances for bromide and chlorinated ethenes and good agreement between measurements of TCE and bromide and model simulations that are predicated on the assumption of core saturation.

Next, the hose clamps and rubber tape were removed to reveal the Teflon sleeve (Fig. B-2h and i). The two ends of the Teflon sleeve were shortened by 0.25 inch to expose the stainless steel so that the end caps could be welded (Fig. B-2j). Each core was then inserted into a custom machined stainless steel pipe so that the core fit as snugly as possible. The ends of the pipe were welded to the ends of the stainless steel caps using tungsten inert gas welding, forming a leak-proof seal (Fig. B-2k). The welding was done at a sufficiently low temperature to avoid any disruption of the core or loss of TCE. The two holes on the top chamber were then connected to Swagelok fittings and polypropylene tubing; next, the chamber was purged with either groundwater or lactate-
amended groundwater that contained resazurin but no TCE (Fig. B-2l). Lastly, the Swagelok fittings were replaced by Mininert valves (Fig. B-2m). The integrity of each core was later tested by placing them in an anaerobic jar and monitoring the headspace of the jar for TCE. None was detected following 8 days of incubation in the jar.

The core microcosms were incubated at room temperature (22-24 °C) in the upright position under quiescent conditions (Figures B-5 and B-6). Approximately once per week, 2 mL of fresh groundwater containing resazurin (and lactate for lactate-amended microcosms) was injected and the volume displaced was collected in a sealed 12 mL serum bottle (Fig. B-7). In order to obtain accurate concentration measurement of the fracture, it was essential for sampling method that there be as little mixing of the freshly added groundwater with the groundwater that came out of the top chamber. Computer simulations and preliminary experiment demonstrated no significant mixing with 2 mL sampling volume and less than 30 seconds sampling time. Before sampling, the serum bottle was pre-sealed inside an anaerobic chamber, so it contained no oxygen. This made it possible to observe the color of the groundwater as it emerged from the upper chamber, to ascertain the redox level in the upper chamber. It was necessary to insert a needle into the headspace of the serum bottle as the groundwater flowed in, to avoid pressurization.
Figure B-2. Photos of the steps used to construct an intact rock core microcosm.
Figure B-3. Schematic representations of the top piece (a and c) and bottom piece (b and d).
Figure B-4. Preparation of bladder accumulator filled with groundwater containing TCE and resazurin.
Figure B-5. Photos of the unamended intact core microcosms.
Figure B-6. Photos of the lactate-amended intact core microcosms.
Figure B-7. Sampling process.
At the end of the experiment, the Mininert valves on the head chambers were replaced by hex-head stoppers. All eleven core microcosms were shipped to the University of Guelph on ice. At Guelph, one port at the top of each microcosm was opened and a sterile 18G 1-1/2" needle and sterile 10 mL syringe were used to retrieve the groundwater from the top chamber. Approximately 12 - 15 mL was removed from each microcosm and placed in a 15 mL polypropylene tube and shipped back to Clemson on ice, where they were used for qPCR analysis. The microcosms were placed in a -80 °C freezer vertically with the two-port side on the top. The intent of freezing was to minimize volatilization losses during removal of the casing and cutting the cores into sections. Microcosms were taken out of the freezer after approximately 4 days and the stainless steel case along with all the PTFE wrappings were removed to expose the rock core. Cores were then cut into three longitudinal sections; each of them was further cut in four quarters. One quarter of the core was crushed and organic compounds were extracted with methanol for VOC analysis at the UoG. One quarter was crushed, dried at 40 °C for one month to a constant weight, and leached with a fixed mass of deionized water; one aliquot was sent to University of Ottawa for ions analysis (by ICP-MS/ICP-ES/IC), and another was sent back to Clemson University for organic acids analysis. One quarter was crushed, wrapped in aluminum foil, placed in a vacuum bag purged with nitrogen, vacuum sealed, and sent back to Clemson for quantification of microbes and dehalogenase enzymes.
B.2. Pathway Experiments

As the results will show, the major fate for lactate added to the rock core microcosms was fermentation to acetate. However, less than a stoichiometric level of acetate was produced (i.e., less than one mole of acetate per mole of lactate). One possible explanation was that lactate and acetate diffused into the core. Another was that acetate was consumed for various processes, such as use as an electron donor for reduction of TCE to cDCE, use as an electron donor for sulfate reduction, production of hydrogen via acetate oxidation, or production of methane. It is also possible that some of the lactate and acetate adsorbed to the rock matrix.

It was unclear from monitoring the groundwater collected from the intact microcosms which of these reactions were occurring. Of particular interest was the fate of acetate, since considerable amounts formed. To better understand these reactions, two additional pathway experiments were prepared. The objective of first experiment was to determine which substrates were used for sulfate reduction. A total of 10 bottles was prepared with groundwater from the site; the measured level of sulfate in the groundwater was ~1 mM. Three were amended with acetate, three with hydrogen, one with lactate, and the remaining three served as controls with no substrate added. All were inoculated (0.5 mL per bottle) with groundwater from the lactate-amended crushed core microcosms in this study.

The objective of second experiment was to determine which substrates support reductive dechlorination and to evaluate the extent of dechlorination. A total of 12 bottles was prepared, each containing anaerobic MSM (with ~1.5 mM sulfate) with one
modification, i.e., yeast extract was replaced by a vitamin stock solution to avoid the use of yeast extract as an electron donor. These bottles were inoculated (5% v/v) with the TCE enrichment culture, which was washed (centrifuged and resuspended in MSM) to remove any remaining substrates. To remove hydrogen present in the headspace of the anaerobic chamber in which the bottles were prepared, they were purged with high purity N₂ (outside the glove box) for 1 min before sealing. Three of the bottles were amended with acetate, three with lactate, and three with hydrogen. The remaining three served as unamended controls. All received TCE.
B.3. **Intact Core Microcosm Results**

Results are presented according to the pairs of microcosms, i.e., unamended #1 (U1) and lactate amended #1 (L1), followed by unamended #2 (U2) and lactate amended #2 (L2), etc. These pairs were prepared with adjacent rock cores, in order to minimize the effect of spatial variability when assessing the impact of biostimulation with lactate.

Analysis of the groundwater and several of the initial weekly samples from the unamended rock core microcosms indicated an absence of organic acids (acetate, lactate, formate, propionate, etc.), so no further measurements were made for these parameters. VC, ethene, acetylene and ethane are not shown in the VOC plots because their levels were too low to be visible.

**Unamended #1 and lactate-amended #1**

VOC and inorganic ion results for Unamended Rock Core #1 (U1) are shown in Figure B-8. The first 2 mL sample was taken approximately 1 hr after the top chamber was flushed with groundwater (free of TCE and bromide). Diffusion of TCE and bromide from the core to the top chamber started immediately, resulting in 0.23 mg/L of TCE (Fig. B-8a, inset) and 0.025 mM of bromide (Fig. B-8b, inset) in the first sample. The next data point on the same day shows a decrease in concentration; this occurred as a consequence of removing 2 mL from the top chamber and replacing it with groundwater that was free of TCE and bromide. The lower concentration at each sampling event was calculated based on the mass of TCE and bromide removed in the 2 mL sample and the volume of water in the top chamber (14.5 mL). All the data points for VOC and inorganic ions were
plotted in the same “measured” to “calculated” manner, giving rise to the “saw-tooth” pattern.

After one week of incubation, the concentration of TCE and bromide increased to 0.95 mg/L and 0.094 mM, respectively (Fig. B-8, insets). This rapid increase was the result of the significant concentration gradient between the groundwater in the top chamber and that inside the pore spaces of the rock. The concentrations in the top chamber gradually decreased as the concentration gradient and rates of diffusion lessened, while the volumetric rate of exchange for water in the top chamber remained constant.

There was no indication of reductive dechlorination of TCE in Unamended Rock Core #1, as cDCE, VC and ethene remained below detection during 594 days of monitoring. The lack of reductive dechlorination is consistent with the observation that the redox level remained above -110 mV (as indicated by the pink color of resazurin) and methane remained below 0.06 mg/L. The trend for bromide was similar to TCE, suggesting that transformation of TCE was limited. The pattern for sulfate indicated that the aqueous phase in the rock core contained higher concentrations than the liquid chamber representing the fracture, such that diffusion of sulfate out of the rock core led to an increase in sulfate above what was present in the infused groundwater (1.5 mM). By the time monitoring ended, the sulfate concentration in the effluent from the top chamber was the same as in the groundwater (i.e., at equilibrium). This also indicated an absence of sulfate reduction, which is consistent with a lack of electron donor.

Results for Lactate-amended Microcosm #1 (L1) are shown in Figure B-9. Similar to the unamended controls, there are two data points for each day. The upper
ones are based on measured concentrations in the 2 mL of groundwater that were
displaced from the top chamber; the lower values were calculated based on the mass of
the compounds removed and replaced with groundwater that either did not contain the
compounds (i.e., TCE, bromide, and organic acids other than lactate) or contained them
at a lower concentration (i.e., sulfate). Lactate is an exception. The upper value was
calculated based on the known concentration of lactate in the 2 mL of groundwater that
was added and the measured concentration in the groundwater that was displaced.

TCE started at 0.35 mg/L and rapidly increased to a peak level of 1.49 mg/L,
followed by a slow decrease. The TCE results through approximately day 250 were
similar to U1 (Fig. B-8a). Thereafter, reductive dechlorination of TCE to cDCE began,
such that by day ~375, TCE remained close to 0.009 mg/L and cDCE increased, until it
peaked (1.05 mg/L) around day 360 and then decreased slowly. The onset of reductive
dechlorination in this core was consistent with a change in the color of the groundwater
samples from pink to clear around day 100, as well as maintenance of a low
concentration of sulfate beyond day 200.

Bromide behaved similarly to U1 (Fig. B-8b), indicative of similar rates of
diffusion from the paired rock samples. Starting at 0.051 mM, bromide increased to
0.157 mM after 1 week, and then decreased gradually. Unlike U1, sulfate consumption
started in L1 around day 90 and continued until it dropped below detection and the
sulfate added weekly in the groundwater was repeatedly consumed.

Lactate consumption started immediately and the concentrations in the upper
chamber decreased accordingly until lactate was close to or below detection by day 50
and weekly additions were repeatedly consumed (Fig. B-9c). Acetate did not accumulate with the initial lactate consumption, possibly because lactate was used up for other processes such as sulfate reduction, or acetate was produced but used for other processes. Acetate started to accumulate around day 100 and then leveled off. In an attempt to stimulate cDCE reduction to VC, the concentration of lactate in the groundwater was increased from 2 to 4 mM on day 196, so that after exchanging groundwater, the concentration of lactate in the top chamber was ~0.55 mM. Initially, this higher dose was completely consumed but then some lactate remained (up to 0.14 mM) after one week of incubation. The increased rate of lactate addition led to a consistent increase in acetate. Another increase in the lactate dose was made on day 307, to 5 mM. This increased the residual level of lactate after each week of incubation to ~0.5 mM and acetate continued to increase. Acetate peaked on day 482 (3.7 mM) and then gradually declined, as did the residual level of lactate. An increase in methane corresponded to the plateau and decrease in acetate. Nevertheless, there was no indication of cDCE reduction to VC or ethene.

Propionate was detected starting on day 377; prior measurements were not made due to problems with the HPLC column (i.e., a “ghost peak” eluted at the same time as propionate). Propionate generally increased during the period of the highest lactate addition rate. The maximum level (0.27 mM) was an order of magnitude lower than the peak for acetate. As will be discussed later, accumulation of propionate is an indication that there was an excess of hydrogen from fermentation of lactate. This suggests that the lack of reductive dechlorination of cDCE was not a consequence of an inadequate
availability of electron donor. The decline in acetate beyond day 500 corresponded to an increase in methane output.

**Unamended #2 and lactate-amended #2**

Figure B-10 shows the VOCs and inorganic ion results for Unamended Rock Core #2 (U2). The TCE concentration in the top chamber increased rapidly from 0.45 mg/L to 2.50 mg/L in 3 weeks and then gradually decreased. Reductive dechlorination of TCE to cDCE started around day 63 and was sustained for the remainder of the incubation period, with cDCE reaching a maximum of 3.10 mg/L on day 233. TCE was never completely consumed, fluctuating between 0.011 and 0.19 mg/L. Of the five unamended rock core microcosms that were monitored, this was the only one that exhibited TCE reduction to cDCE. This behavior was consistent with the previous unamended crushed rock microcosms, some of which exhibited TCE reduction to cDCE and others did not (Table 3.1). The color of the groundwater samples from this microcosm fluctuated between clear and light pink (Fig. B-10a); reductive dechlorination of TCE occurred in spite of the higher redox level than in the lactate-amended microcosms. This is consistent with the hypothesis that *Geobacter* were responsible for TCE reduction to cDCE, since they can tolerate a somewhat higher redox level than *Dehalococcoides* (197). Barlett et al. (198) reported little to no competition between *Geobacter* and sulfate reducing bacteria when acetate was abundant. There was no detection of VC or ethene in U2, and methane levels remained low, as in U1.

The trend for bromide in U2 (Fig. B-10b) was similar to U1, but the level was more than two times higher than in U1. This reflected a higher diffusion rate in U2 than
U1. Sulfate remained at relatively constant levels in U2, suggesting that the concentration in the pore water in the rock and the groundwater were similar, and that no significant level of sulfate reduction occurred.

Results for Lactate-amended Microcosm #2 (L2) are shown in Figure B-11. As in L1, TCE initially rose sharply and then decreased gradually, until the onset of reductive dechlorination around day 91. Thereafter, cDCE accumulated to a peak of 2.97 mg/L on day 223, followed by a gradual decline. VC and ethene were not detected. Unlike L1, methane remained at the low levels found in the unamended microcosms, and there was no significant decrease in acetate. Sulfate was gradually reduced as lactate was consumed, with weekly increases attributable to the addition of site groundwater containing approximately 1.5 mM sulfate. Bromide followed similar patterns to L1, although the level was two to three times higher, for the same reason as U2 and U1.

As in L1, lactate in L2 was consumed until it was no longer present in the upper chamber except after its addition with the groundwater (Fig. B-11c). Unlike L1, there was no significant accumulation of lactate in response to the step increases in lactate in the groundwater added to the upper chamber (i.e., from 2 to 4 to 5 mM on days 196 and 307, respectively). Acetate accumulated gradually, especially after the increase in lactate addition. Propionate increased steadily after day ~430, likely a consequence of an increased availability of hydrogen from fermentation of higher doses of lactate.

Unamended #3 and lactate-amended #3

Figure B-12 shows the VOC and inorganic ion results for Unamended Rock Core #3 (U3). Similar to U1, there was no detectable reductive dechlorination of TCE to cDCE.
TCE increased from 0.36 mg/L to a peak level of 2.40 mg/L within 2 weeks, and then decreased steadily. Methane remained below 0.07 mg/L. Between days 70 and 280, the color of the groundwater samples fluctuated between clear or light pink, but after that the color became consistently pink, consistent with a lack of electron donor. The trends for bromide and sulfate were very similar to those for U2. The peak bromide level (0.28 mM) was reached on day 62 and then decreased in accordance with diffusion out of the rock. Sulfate was close to the values present in the groundwater.

Results for Lactate-amended Microcosm #3 (L3) are shown in Figure B-13. This was the only lactate-amended microcosm that did not exhibit significant levels of TCE reduction to cDCE, in spite of the fact that the lactate was consumed, with accumulation of acetate and, to a lesser extent, propionate. The pattern of lactate consumption was similar to L1, i.e., not all of the lactate added was consumed. TCE started at 0.42 mg/L and rapidly increased to a peak of 2.57 mg/L, followed by a slow decrease. The color of the groundwater samples went clear after day 140, indicating that the redox conditions were conducive to organohalide respiration. The change in color coincided with the onset of sulfate reduction. At the same time, there was only a minor increase in cDCE, to a maximum of 0.079 mg/L; this was higher than in U3 (0.015 mg/L), but still well below what accumulated in L1 and L2. Methane started to accumulate around day 300, indicative of an excess of electron donor and the absence of sulfate inhibition. The lack of reductive dechlorination was most likely a consequence of a lack of the necessary dechlorinating microbes. While such microbes appear to be common at this site, they are apparently not uniformly distributed; hence the lack of response to biostimulation in L3.
The bromide profile in L3 was similar to that in U3. Bromide increased from an initial level of 0.059 mM to a peak of 0.36 mM in 1 week, followed by a slow decrease.

*Unamended #4 and lactate-amended #4*

Unamended Rock Core #4 (U4) broke during preparation so no results are shown. Results for Lactate-amended Microcosm #4 (L4) are shown in Figure B-14. VOC results in L4 resemble those in L2. TCE started at 0.72 mg/L and rapidly increased to a peak level of 3.19 mg/L, followed by a relatively slow decrease. The pink color of the resazurin faded by day 100, by which time there was no sulfate remaining in the upper chamber. Soon thereafter, TCE reduction to cDCE was underway. However, there was a modest rebound in TCE between days 189 and 250 and cDCE accumulation began to level off. TCE subsequently decreased again while cDCE accumulation resumed, then plateaued around day 290, and then started to decrease after day 380. Methane began to accumulate after approximately day 500.

The bromide concentration profile was similar to that in U3; it increased from an initial level of 0.059 mM to a peak level of 0.36 mM in 1 week, followed by a gradual decline. Lactate consumption was complete except for a brief interval around day 200, which preceded the modest rebound in TCE. Acetate began to accumulate once sulfate consumption was complete. Propionate accumulation leveled off just as acetate did, but at an order of magnitude lower concentration.

*Unamended #5 and lactate-amended #5*

Results for this pair can be found in the main text.

*Unamended #6 and lactate-amended #6 and summary*
Figure B-15 shows the VOC and inorganic ion results for Unamended Rock Core #6 (U6). Similar to U1 and U3, there was no detectable reductive dechlorination of TCE to cDCE. TCE increased from 0.290 mg/L to a maximum of 2.476 mg/L during the first few weeks, and then decreased steadily. Methane remained below 0.09 mg/L. The color of the groundwater samples remained pink, consistent with a lack of electron donor. The trends for bromide and sulfate in U6 were very similar to those for U3 and U5. The peak bromide level (0.25 mM) was reached in two weeks and then decreased in accordance with diffusion out of the rock. Sulfate was close to the values present in the groundwater.

Results for Lactate-amended Microcosm #6 (L6) are shown in Figure B-16. As in L2 and L5, TCE initially rose sharply and then decreased gradually, until the onset of reductive dechlorination around day 91. Thereafter, cDCE accumulated to a peak of 3.39 mg/L on day 223, followed by a gradual decline. VC and ethene were not detected. Methane remained low, as in L2 and L5. Sulfate was gradually reduced as lactate was consumed, with weekly increases attributable to the addition of site groundwater containing approximately 1.5 mM sulfate. Bromide followed similar patterns to L2 through L5.

As in L2, L4 and L5, lactate consumption was complete or nearly so after each weekly addition to the head chamber (Fig. B-16c). There was no significant accumulation of lactate in response to the step increases. Acetate accumulated gradually over time, especially after the increases in lactate addition. Propionate increased steadily after day ~380, likely a consequence of lactate reduction in response to an increased availability of hydrogen.
Figure B-8. Results for (a) VOCs and (b) inorganic ions in unamended intact rock core microcosm U1. The color bar below the legend in (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-9. Results for (a) VOCs, (b) inorganic ions, and (c) organic acids in lactate-amended intact rock core microcosm L1. The color bar below the legend in panel (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-10. Results for (a) VOCs and (b) inorganic ions in unamended intact rock core microcosm U2. The color bar below the legend in (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-11. Results for (a) VOCs, (b) inorganic ions, and (c) organic acids in lactate-amended intact rock core microcosm L2. The color bar below the legend in panel (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-12. Results for (a) VOCs and (b) inorganic ions in unamended intact rock core microcosm U3. The color bar below the legend in (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-13. Results for (a) VOCs, (b) inorganic ions, and (c) organic acids in lactate-amended intact rock core microcosm L3. The color bar below the legend in panel (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-14. Results for (a) VOCs, (b) inorganic ions, and (c) organic acids in lactate-amended intact rock core microcosm L4. The color bar below the legend in panel (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-15. Results for (a) VOCs and (b) inorganic ions in unamended intact rock core microcosm U6. The color bar below the legend in (a) indicates the status of the resazurin in weekly groundwater samples.
Figure B-16. Results for (a) VOCs, (b) inorganic ions, and (c) organic acids in lactate-amended intact rock core microcosm L6. The color bar below the legend in panel (a) indicates the status of the resazurin in weekly groundwater samples.
B.4. Cumulative TCE and cDCE Removal Over Time

The cumulative amount of TCE and cDCE removed from the microcosms (expressed in µmoles) as a consequence of the weekly exchange of groundwater is shown in Figures B-17 and B-18. Six panels are shown, one for each unamended and lactate-amended pair of microcosms.

Microcosms U1 and L1 behaved similarly until day ~300, when the rate of reductive dechlorination of TCE to cDCE accelerated in the lactate-amended microcosm (Fig. B-17a). Even though both microcosms presumptively had the same initial mass of TCE, the onset of reductive dechlorination in L1 led to a higher rate of removal of the chlorinated ethenes. This is likely because cDCE diffuses at a higher rate and does not adsorb as strongly as TCE, allowing more mass to enter the upper chamber and be removed.

Reductive dechlorination occurred at similar rates in microcosms U2 and L2. Consequently, the cumulative amount removed from this pair was similar (Fig. B-17b). There was no significant reductive dechlorination in microcosms U3 and L3 (Fig. B-17c); cumulative removal from this pair was also similar and less than one-half of the total removal from U2 and L2. Only one microcosm is shown for the fourth pair, since U4 broke.

In the last two pairs (U5 and L5 in Fig. B-18b; U6 and L6 in Fig. B-18c), the behavior was similar to L1 and U1 (Fig. B-17a) since reductive dechlorination occurred in the lactate–amended microcosms but not in the unamended microcosms.
Figure B-17. Cumulative TCE and cDCE removal from intact rock core microcosms, (a) pair #1, (b) pair #2 and (c) pair #3.
Figure B-18. Cumulative TCE and cDCE removal from intact rock core microcosms, (a) pair #4, (b) pair #5 and (c) pair #6.
B.5. Stainless Steel Vessel Controls

The intact cores were accompanied by three container controls, i.e., stainless steel cylinders of the same dimensions as those used for the intact cores, but containing only groundwater with TCE, bromide and resazurin. The main function of these cylinders was to serve as controls for the $\delta^{13}$C measurements. However, TCE levels were also monitored over time, on six occasions. When 2 mL samples were removed for analysis of TCE, the volume removed was replaced with 2 mL of groundwater without TCE. A mass balance on TCE was calculated based on the mass present at the end of monitoring, plus the mass removed during sampling, divided by the mass initially present. The average recovery of TCE was 67%±5%. The losses were a consequence of leakage. The cylinders used were preliminary versions that experienced some problems with the welds. While it would have been preferable to have leak-proof container controls, this shortcoming was considered acceptable since their main function was for comparison to the $\delta^{13}$C data from the intact rock core microcosms.
B.6. Methane and Acetylene

Methane concentrations are shown in Figure B-19 for microcosm pairs 1-3, and in Figure B-20 for pairs 4-6. There was no accumulation of methane in any of the unamended microcosms. This is consistent with the presumption that these microcosms were deficient in electron donor. Methane accumulation occurred in L1, L3, and L4, but only after at least 400 days of incubation. This is consistent with lactate addition in excess of what was needed for sulfate reduction, at least based on the amount of sulfate added in the weekly groundwater exchanges. Sulfate within the cores may have created an additional demand for lactate and limited methane production. As mentioned above, reductive dechlorination and propionate accumulation represented a minor part of the total electron donor demand.

Acetylene concentrations are shown in Figure B-21a for the unamended microcosms and in Figure B-21b for the lactate-amended ones. Acetylene levels remained below 0.02 mg/L in the unamended microcosms, with periodic increases and decreases. The reason for the decreases is not known but may be related to anaerobic biodegradation or diffusive loss. Acetylene levels were consistently higher in the lactate-amended microcosms. An electron donor is necessary for abiotic degradation of TCE and cDCE to acetylene, as it is for reductive dechlorination. Notably, acetylene levels were highest in L1 and L3, which exhibited the lowest levels of reductive dechlorination (Fig. B-21b). Furthermore, acetylene levels started to increase in these microcosms around the same time that methane started to accumulate (Fig. B-19) at around day 400.
Figure B-19. Methane production in intact rock core microcosms, (a) pair #1, (b) pair #2 and (c) pair #3.
Figure B-20. Methane production in intact rock core microcosms, (a) pair #4, (b) pair #5 and (c) pair #6. Unlike other pairs, pair #4 only has one microcosm (L4).
Figure B-21. Acetylene levels in intact rock core microcosms (a) unamended controls, (b) lactate-amended microcosms.
B.7. Lactate, Sulfate Consumption, and Acetate Production

Based on weekly monitoring data for organic acids and sulfate, the cumulative amounts of lactate and sulfate consumed and acetate formed were calculated. Figure B-30 shows the results for L1, L2, and L3; Figure B-31 shows the results for L4, L5, and L6. Since lactate was not added to the unamended microcosms, data for these microcosms were not included in the comparison.

Cumulative consumption of lactate (red circles) was calculated as the cumulative lactate added minus the lactate remaining in the chamber when groundwater was exchanged each week. Cumulative acetate production (green diamonds) was calculated by summing the acetate removed from the top chamber. Since this does not consider the acetate consumed via biodegradation or the amount that diffused into the core, it is likely an underestimate. Cumulative sulfate consumption (blue triangle) was calculated by subtracting the sulfate level in the top chamber when groundwater was exchanged each week from the amount of sulfate added via the fresh groundwater. This does not consider the amount of sulfate that diffused in or out of the core. Since the sulfate level kept decreasing in the upper chamber, diffusion was presumably favored in the direction of the core to the chamber, so that sulfate consumption across the full microcosm was likely underestimated.

The predominant metabolic activity was sulfate reduction associated with partial oxidation of lactate to acetate. However, the COD associated with acetate production and sulfate consumption was less than half of the theoretical lactate COD, suggesting the occurrence of other COD utilizing processes, e.g., methane and propionate production,
chlorinated ethene reduction, and microbial growth. Accumulation of propionate and methane are not shown in Figures B-30 and B-31, but they represented a minor percentage of the electron flow from lactate, as did the electron equivalents consumed for reduction of TCE to cDCE.
Figure B-22. Consumption of lactate and sulfate and production of acetate in intact rock core microcosms (a) L1, (b) L2 and (c) L3.
Figure B-23. Consumption of lactate and sulfate and production of acetate in intact rock core microcosms (a) L4, (b) L5 and (c) L6.
B.8.  **pH Results and Mass Increase**

Table B-1 summarizes the pH and mass increase in all microcosms. Figures B-30 and B-31 show the pH level in weekly samples removed from the top chamber for the five unamended microcosms. The range was 7.26 to 8.03; the average pH levels for U1, U2, U3, U5 and U6 were 7.83, 7.74, 7.73, 7.80 and 7.90, respectively. Figures B-30 and B-31 show the pH level in weekly samples removed from the top chamber for the six lactate-amended microcosms. The range was 6.99 to 8.13; average pH levels for L1, L2, L3, L4, L5 and L6 were 7.55, 7.58, 7.51, 7.59, 7.60 and 7.73, respectively.

Mass changes are presented in Figures B-30 and B-31 for the unamended microcosms and B-30 and B-31 for the lactate-amended microcosms. Monitoring the total mass served two purposes: 1) weighing the microcosms before and after the sampling process ensured that the sample collected was properly displaced by the groundwater injected; and 2) long-term monitoring was a quick way to reveal if a leak developed.

All of the microcosms experienced minor increases in mass. This was likely a consequence of displacing a gas bubble; on several occasions, less than 2 mL of groundwater was collected even though 2 mL was added.

In microcosm L4 (Fig. B-31a), there was an abrupt decrease in mass of ~ 0.5 g around day 460. This was caused by an unclosed Mininert sampling valve, which allowed a small amount of water and VOC to leak out. Subsequent monitoring did not reveal any significant consequences other than this minor mass loss.
Table B-1. Average pH level and mass gain in the intact core microcosms.

<table>
<thead>
<tr>
<th>Microcosm</th>
<th>Average pH</th>
<th>Mass increase (g)</th>
<th>Saturation level (%) &lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1</td>
<td>7.83</td>
<td>3.8</td>
<td>86.4%</td>
</tr>
<tr>
<td>L1</td>
<td>7.55</td>
<td>3.2</td>
<td>88.5%</td>
</tr>
<tr>
<td>U2</td>
<td>7.74</td>
<td>2.4</td>
<td>91.6%</td>
</tr>
<tr>
<td>L2</td>
<td>7.58</td>
<td>1.9</td>
<td>93.3%</td>
</tr>
<tr>
<td>U3</td>
<td>7.73</td>
<td>3.1</td>
<td>89.0%</td>
</tr>
<tr>
<td>L3</td>
<td>7.51</td>
<td>2.8</td>
<td>89.9%</td>
</tr>
<tr>
<td>L4</td>
<td>7.59</td>
<td>1.3</td>
<td>95.3%</td>
</tr>
<tr>
<td>U5</td>
<td>7.80</td>
<td>3.6</td>
<td>87.1%</td>
</tr>
<tr>
<td>L5</td>
<td>7.60</td>
<td>4.5</td>
<td>84.0%</td>
</tr>
<tr>
<td>U6</td>
<td>7.90</td>
<td>5.4</td>
<td>80.9%</td>
</tr>
<tr>
<td>L6</td>
<td>7.73</td>
<td>2.3</td>
<td>91.8%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Saturation level is calculated by deducting the mass increase from theoretical pore water mass (28 g), then dividing it with the theoretical pore water mass.
Figure B-24. pH levels in intact rock core microcosms (a) U1, (b) U2 and (c) U3.
Figure B-25. pH levels in intact rock core microcosms (a) U5 and (b) U6.
Figure B-26. pH levels in intact rock core microcosms (a) L1, (b) L2 and (c) L3.
Figure B-27. pH levels in intact rock core microcosms (a) L4, (b) L5 and (c) L6.
Figure B-28. Weight change of intact rock core microcosms (a) U1, (b) U2 and (c) U3.
Figure B-29. Weight change of intact rock core microcosms (a) U5 and (b) U6.
Figure B-30. Weight change of intact rock core microcosms (a) L1, (b) L2 and (c) L3.
Figure B-31. Weight change of intact rock core microcosms (a) L4, (b) L5 and (c) L6.
B.9. End of Incubation Evaluation

When incubation ceased, the cores were shipped to the University of Guelph for destructive sampling. The cores were then processed so that they could be analyzed for VOCs, bromide, chloride, organic acids, and microbes.

Bromide results are shown in the main text and chloride results are shown in Figure B-32. The background level of chloride was more than 10 fold higher than the amount of chloride potentially released from complete reduction of TCE to cDCE. In addition, chloride-containing groundwater was constantly fed to the microcosms during the sampling process. Therefore, comparisons of chloride concentrations among the different core microcosms does not provide meaningful evidence for reductive dechlorination activity. A Student’s $t$-test comparing the concentrations of chloride in the chamber of microcosms with and without reductive dechlorination showed a statistically significant difference. However, there was no statistically significant difference in the amounts of chloride in the rock sections or the total amount in the whole microcosm (Table B-2).
Figure B-32. Results for end-of-incubation analyses of chloride in the intact core microcosms. The four bars for each microcosms represent (from left to right) the concentrations in head chamber, in pore water inside top section, middle section and bottom section, annotated as C, T, M and B, respectively.

Table B-2. Student’s $t$-test results for chloride concentration between cores with reductive dechlorination (w/ RD) and without reductive dechlorination (w/out RD) in end of incubation analysis.

<table>
<thead>
<tr>
<th>Location</th>
<th>Bromide</th>
<th>w/ RD</th>
<th>w/out RD</th>
<th>$p$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber</td>
<td>Average (mg/L)</td>
<td>0.50</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stdev (mg/L)</td>
<td>0.0001</td>
<td>0.0004</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>Sample #</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Core slices</td>
<td>Average (mg/L)</td>
<td>0.52</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stdev (mg/L)</td>
<td>0.004</td>
<td>0.002</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Sample #</td>
<td>18</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Average (mg/microcosm)</td>
<td>2.06</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stdev (mg/microcosm)</td>
<td>0.04</td>
<td>0.01</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Sample #</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

$^a H_0: \mu_{w/ RD} \geq \mu_{w/out RD}$. 
Results for organic acids are shown in Figure B-33 for lactate and formate. Lactate concentration was close to or below detection. Formate was close to or below detection in the head chamber but was detectable in most of the core sections, at concentration up to 1.2 mM. The measurement of organic acids was performed with samples of the cores that were crushed, dehydrated and rehydrated, a process that may have resulted in formation of formate, which may explain the discrepancy between the chamber and core sections.

Results for the end of incubation qPCR analysis are shown in Figures B-34 and B-35. DNA was extracted from groundwater in the head chamber or the core section, using ~1 g crushed sample from each sliced section. The analyses included *Dehalococcoides, vcrA, bvCA, tceA, Geobacter, Geobacter lovleyi*, and total bacteria. However, only *Geobacter* and total bacteria were amplified during qPCR.

A Student’s t-test was performed on the amount of *Geobacter* and total bacteria in the lactate-amended and unamended microcosms. The mean values for *Geobacter* and total bacteria in the head chambers were higher in the lactate-amended microcosms (p<0.05, one-tail) but there was no difference for the core sections. qPCR results for the core sections exhibited lots of variations, possibly due to the heterogeneity of microbial distribution and the small sampling quantity (i.e., 1 g crushed rock) from each section.
Figure B-33. Results for end-of-incubation analyses of (a) lactate and (b) formate in the intact core microcosms. The four bars for each microcosms represent (from left to right) the concentrations in head chamber, and in pore water inside top section, middle section and bottom section, annotated as C, T, M and B, respectively.
Figure B-34. Results for end-of-incubation qPCR analyses for core pair #1 (panel a, b), #2 (c, d) and #3 (e, f). Data were presented as total gene copies per section, including the water chamber (W), top rock section (T), middle rock section (M) and bottom rock section (B). Bars with solid and tile patterns indicate existence and absence of reductive dechlorination, respectively.
Figure B-35. Results for end-of-incubation qPCR analyses for core pair #4 (panel a, b), #5 (c, d) and #6 (e, f). Data were presented as total gene copies per section, including the water chamber (W), top rock section (T), middle rock section (M) and bottom rock section (B). Bars with solid and tile patterns indicate existence and absence of reductive dechlorination, respectively.
B.10. Crushed Rock Microcosms

As described previously, crushed rock microcosms were prepared at the same time as the intact rock core microcosms with rock from an adjoining part of the same borehole from which cores were obtained for the intact microcosms. The intent of the crushed rock microcosms was to have a direct comparison to the intact cores.

Representative results for the unamended microcosms that were prepared with ~0.7 mg/L of TCE are shown in Figure B-36, and for the microcosms prepared with ~14 mg/L in Figure B-37. In two of the six replicates that received ~0.7 mg/L, TCE underwent a minor amount or a relatively slow rate of reductive dechlorination to cDCE. Only one of the six microcosms that started with ~14 mg/L exhibited a slow rate of reductive dechlorination to cDCE.

Representative results for the lactate-amended microcosms that were prepared with ~0.7 mg/L of TCE are shown in Figure B-38. All six replicates exhibited reductive dechlorination to cDCE and one exhibited complete reduction to ethene. Representative results for the lactate-amended microcosms that were prepared with ~14 mg/L of TCE are shown in Figure B-39. Three of the six replicates exhibited reductive dechlorination to cDCE and only one exhibited a low level of VC but no ethene. This suggested that the higher initial concentration of TCE may have an inhibitory impact on the onset of reductive dechlorination.

The fact that only one of the 12 lactate-amended microcosms exhibited ethene formation suggests the rock used in this study contained a low level of *Dehalococcoides* and they are heterogeneously distributed. The fact that none of the high concentration
crushed rock microcosms exhibited dechlorination of cDCE was consistent with the absence of reductive dechlorination beyond cDCE in the intact rock core microcosms, which were infused with TCE at a similar initial concentration.

Figure B-36. Representative VOC results for the unamended crushed rock microcosms prepared with ~0.7 mg/L of TCE (a) that exhibited no reductive dechlorination; and (b) that exhibited reductive dechlorination to cDCE.
Figure B-37. Representative VOC results for the unamended crushed rock microcosms prepared with ~14 mg/L of TCE (a) that exhibited no reductive dechlorination; and (b) that exhibited reductive dechlorination to cDCE.
Figure B-38. Representative VOC results for the lactate-amended crushed rock microcosms prepared with ~0.8 mg/L of TCE (a) that exhibited reductive dechlorination only to cDCE; and (b) that exhibited reductive dechlorination to ethene.
Figure B-39. Representative VOC results for the lactate-amended crushed rock microcosms prepared with ~14 mg/L of TCE (a) that exhibited no reductive dechlorination; and (b) that exhibited reductive dechlorination to cDCE and a trace level of VC (too low to be visible).
B.11. Fate of Lactate, Acetate and Hydrogen

The results from previous study clearly demonstrated that addition of lactate enhanced complete reductive dechlorination of TCE to ethene. It was not established in the previous study if the enhancement was a direct consequence of lactate or one of its metabolic products, i.e., acetate and hydrogen. It was also not clear which of these electron donors supported sulfate reduction and methanogenesis. Additional pathway experiments were therefore performed to ascertain if acetate could serve as the electron donor for reductive dechlorination and sulfate reduction, or if hydrogen supported sulfate reduction; hydrogen’s role in reductive dechlorination is well established. The fate of acetate was of particular interest, since it accumulated in the head chamber of the intact rock core microcosms and diffused into the cores.

Table B-3 summarizes the design for the two experiments performed to evaluate the pathways for electron donor utilization. For the first experiment, the focus was on which of the electron donors supported sulfate reduction. Aliquots from the crushed rock microcosms in this study were added to groundwater, which contained a background level of sulfate. The treatments evaluated were unamended (negative control), lactate-amended (positive control), acetate-amended, and hydrogen-amended. Overall results for the number of bottles that exhibited sulfate reduction, methane production, acetate production, TCE reduction and cDCE reduction are shown in Table B-4. Results for cumulative consumption of sulfate, acetate, lactate, and hydrogen are shown in Figure B-40. Lactate stimulated sulfate reduction first. The rate of sulfate reduction paralleled the rate at which lactate was consumed (Fig. B-40b). The plateau in sulfate consumption
between days 19 and 42 corresponded to a period when lactate was not added. Once the available sulfate was depleted after ~day 61, lactate consumption continued but at a slower rate. Approximately one mole of acetate was produced per mole of lactate consumed (data not shown); a minor level of propionate was also produced, i.e., less than 10% of the molar amount of acetate. Sulfate reduction was also supported by H₂, at a slower rate than lactate (Fig. B-40a). Approximately 7 moles of hydrogen were consumed per mole of sulfate removed, which is higher than the stoichiometric amount needed (4:1); the fate of the excess hydrogen was not evaluated. Only one of the three acetate-amended microcosms exhibited sulfate consumption (Table B-4); cumulative results for this bottle are shown in Figure B-40 (hence the lack of error bars). There was a lag in the onset of sulfate reduction in this bottle but eventually it caught up with hydrogen. Approximately 2 moles of acetate were consumed per mole of sulfate, which is twice as high as the stoichiometric amount. In the other two microcosms, acetate and sulfate were not consumed. Since only one of the three acetate-amended microcosms exhibited sulfate reduction, the results of this experiment left it unclear if acetate supported sulfate reduction.

For the second experiment, the focus was on which of the electron donors supported reductive dechlorination of TCE. Since groundwater was used and sulfate was present, the second experiment also explored which of the donors supported sulfate reduction. Aliquots from a previous established enrichment culture that reduced TCE to ethene were used to inoculate basal medium supplemented with a vitamin solution (Table B-3). The medium used for the enrichment cultures was prepared with yeast extract to
provide vitamins. However, in a preliminary experiment with only medium (intended to serve as the negative control), TCE was readily dechlorinated, indicating that yeast extract was also an effective electron donor. In the subsequent experiment, a vitamin solution replaced the yeast extract. The treatments evaluated were unamended (negative control), lactate-amended (positive control), acetate-amended, and hydrogen-amended.

Figure B-41 summarizes the sulfate consumption and electron donor consumption. Consistent with the first pathway experiment, lactate and hydrogen supported sulfate reduction. In this experiment, none of the acetate-amended bottles supported sulfate reduction; the weight of evidence indicates acetate does not fill this role in samples from the site. The downward drift in the sulfate levels shown in Figure B-41a for the unamended and acetate-amended treatment is a consequence of repeated sampling and replacement of medium in the bottles to resupply TCE; the medium added did not contain sulfate, so the amount remaining was diluted over time. One noteworthy difference between the two pathway experiments was the greater amount of propionate that accumulated in the lactate-amended bottles for the second one. Approximately 0.33 mole of acetate and 0.67 mole of propionate were formed per mole of lactate consumed (data not shown). Propionate accumulation was greatest after sulfate was consumed, suggesting that excess electron donor supported reduction of lactate to propionate. Formate also accumulated (data not shown), which likely underwent disproportionation to CO₂ and hydrogen.

Figure B-42 summarizes cumulative TCE consumption, VC production and methane production for the second pathway experiment. Reductive dechlorination of
TCE to cDCE was supported by lactate, acetate and hydrogen, while no dechlorination occurred in the unamended control bottles (Fig. B-42a). TCE consumption started almost immediately in the lactate and acetate-amended treatments while there was a lag in the hydrogen-amended bottles. TCE (10 µmol/bottle) was added once per week, during which time it was fully consumed. Production of VC was also observed with each electron donor, with lactate being the most effective (Fig. B-42b). At the time the experiment was terminated, ethene production had not yet started and there was no methane formation (Fig. B-42c). The fact that acetate supported dechlorination of cDCE was unexpected, since the *Dehalococcoides* that mediate this reaction are only known to use hydrogen as their electron donor; presumably the acetate was oxidized to CO$_2$ and hydrogen.

Table B-5 summarizes the potential reactions in the microcosms. Lactate and hydrogen supported sulfate reduction, while acetate did not. Hydrogen, lactate, and acetate supported reductive dechlorination of TCE to cDCE and VC. It is likely that acetate supported reductive dechlorination of cDCE to VC via its oxidation to CO$_2$ and hydrogen; there was some evidence in support of hydrogen accumulation in the acetate amended treatment. This has implications for the intact core microcosms, since the acetate that formed from lactate in the upper chamber diffused into the rock, where it may have served as an electron donor. Propionate formed from lactate; it is likely that subsequent fermentation of propionate yielded hydrogen that supported reductive dechlorination and sulfate reduction. Formate also formed from lactate and was likely converted to CO$_2$ and hydrogen.
Figure B-40. Results for the first pathway experiment, based on (a) sulfate consumption, and (b) the amount of electron donors consumed in corresponding treatments (i.e., acetate in the acetate treatment, lactate in the lactate treatment, H2 in the H2 treatment). Error bar indicates the standard deviation. Single bottles are shown for lactate, and the one in three acetate-amended bottles that showed sulfate reduction.
Figure B-41. Results for the second pathway experiment, based on (a) sulfate consumption; and (b) the amount of electron donors consumed in corresponding treatments. Each data point is the average of triplicate bottles per treatment; error bars represent standard deviations.
Figure B-42. VOC results for the second pathway experiment, in terms of (a) TCE consumption; (b) VC production; and (c) methane production. Each data point is the average of triplicate bottles per treatment (UN, Acetate, Lactate, and Hydrogen); error bars represent standard deviations.
Table B-3. Summary of experimental conditions for the pathway experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundwater mixture</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Medium + vitamin stock</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Contains sulfate</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>TCE addition</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Inoculum</td>
<td>crushed rock microcosm + lactate</td>
<td>enrichment culture fed with TCE</td>
</tr>
</tbody>
</table>

Table B-4. Summary of outcomes for the pathway experiments.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UN</td>
<td>Acetate</td>
<td>Lactate</td>
<td>$H_2$</td>
<td>UN</td>
</tr>
<tr>
<td>Sulfate reduction</td>
<td>0/3$^a$</td>
<td>1/3</td>
<td>1/1</td>
<td>3/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Methane production</td>
<td>1/3</td>
<td>1/3</td>
<td>1/1</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Acetate production</td>
<td>3/3$^b$</td>
<td>-</td>
<td>1/1</td>
<td>3/3$^b$</td>
<td>0/3</td>
</tr>
<tr>
<td>TCE reduction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/3</td>
</tr>
<tr>
<td>VC production</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/3</td>
</tr>
</tbody>
</table>

$^a$ Numerators indicate the number of bottles that exhibited the activity and denominators indicate the total number of replicates.

$^b$ Acetate was likely produced from the lactate introduced into the UN and $H_2$ experiments via the inoculum; acetate reached a similar level as the initial lactate.
Table B-5. Potential reactions in microcosms amended with lactate.

<table>
<thead>
<tr>
<th>#</th>
<th>Reaction</th>
<th>Evidence</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₂HCl₃ + H₂ → C₂H₂Cl₂ + H⁺ + Cl⁻</td>
<td>√</td>
<td>(199-200)</td>
</tr>
<tr>
<td>2</td>
<td>C₂H₂Cl₂ + H₂ → C₂H₃Cl + H⁺ + Cl⁻</td>
<td>√</td>
<td>(199-200)</td>
</tr>
<tr>
<td>3</td>
<td>C₂H₃Cl + H₂ → C₂H₄ + H⁺ + Cl⁻</td>
<td>√</td>
<td>(199-200)</td>
</tr>
<tr>
<td>4</td>
<td>C₂HCl₃ + 0.25CH₃COO⁻ + 0.5H₂O → C₂H₂Cl₂ + 0.75H⁺ + 0.5CO₂ + Cl⁻</td>
<td>√</td>
<td>(201)</td>
</tr>
<tr>
<td>5</td>
<td>CH₃COO⁻ + SO₄²⁻ + 2H⁺ → 2CO₂ + 2H₂O + HS⁻</td>
<td>×</td>
<td>(202)</td>
</tr>
<tr>
<td>6</td>
<td>CH₃COO⁻ + H⁺ → CH₄ + CO₂</td>
<td>?</td>
<td>(204)</td>
</tr>
<tr>
<td>7</td>
<td>CH₃COO⁻ + H⁺ + 2H₂O → 2CO₂ + 4H₂</td>
<td>?</td>
<td>(204)</td>
</tr>
<tr>
<td>8</td>
<td>CH₃CHOHCOO⁻ + H₂O → CH₃COO⁻ + CO₂ + 2H₂</td>
<td>√</td>
<td>(202, 205)</td>
</tr>
<tr>
<td>9</td>
<td>CH₃CHOHCOO⁻ → 0.33CH₃COO⁻ + 0.66CH₃CH₂COO⁻ + 0.33CO₂ + 0.33H₂O</td>
<td>√</td>
<td>(206)</td>
</tr>
<tr>
<td>10</td>
<td>CH₃CH₂COO⁻ + 2H₂O → CH₃COO⁻ + CO₂ + 3H₂</td>
<td>?</td>
<td>(203)</td>
</tr>
<tr>
<td>11</td>
<td>CH₃CHOHCOO⁻ + 0.5SO₄²⁻ + 0.5 H⁺ → CH₃COO⁻ + CO₂ + H₂O + 0.5HS⁻</td>
<td>√</td>
<td>(202)</td>
</tr>
<tr>
<td>12</td>
<td>CH₃CHOHCOO⁻ → CH₃CHO + HCOO⁻</td>
<td>?</td>
<td>(207)</td>
</tr>
<tr>
<td>13</td>
<td>CH₃CHO + NAD⁺ + H₂O → CH₃COO⁻ + 2H⁺ + NADH</td>
<td>?</td>
<td>(208)</td>
</tr>
<tr>
<td>14</td>
<td>HCOO⁻ + H⁺ → H₂ + CO₂</td>
<td>?</td>
<td>(209)</td>
</tr>
<tr>
<td>15</td>
<td>4H₂ + 2CO₂ → CH₃COO⁻ + H⁺ + 2H₂O</td>
<td>√</td>
<td>(202, 205,</td>
</tr>
<tr>
<td>16</td>
<td>4H₂ + CO₂ → CH₄ + 2H₂O</td>
<td>×</td>
<td>(202-203,</td>
</tr>
<tr>
<td>17</td>
<td>4H₂ + SO₄²⁻ + H⁺ → HS⁻ + 4H₂O</td>
<td>√</td>
<td>(202, 205)</td>
</tr>
<tr>
<td>18</td>
<td>4HCOO⁻ + 4H⁺ → CH₄ + 3CO₂ + 2H₂O</td>
<td>?</td>
<td>(210)</td>
</tr>
</tbody>
</table>

*a* "√" indicates strong evidence in support of the reaction.

*b* "×" indicates no evidence found in support of the reaction.

*c* "?" indicates insufficient evidence to either reject or support the reaction.
C. Supplementary Material for Chapter 4

C.1. Model Setup

A “Time Dependent” study was created with a built-in model in COMSOL called “Species Transport in Porous Media (chpm)”. The head chamber was assigned a property referred to as “free flow” since it contains only groundwater; the rock was assigned a property called “mobile fluid, immobile solid”, specifically designed for porous media. The initial concentrations of TCE and bromide within the pore spaces of the rock were set at 20 mg/L (152 µM) and 1 mM, respectively. The initial concentrations of TCE and bromide in the simulated fracture (i.e., the head chamber) were set to zero.

C.2. Comparison Between Continuous and Weekly Models

Preliminary results from the weekly model and continuous model approaches show good consistency with each other (Fig. C-1), and fit the experimental data for TCE, cDCE and bromide in the intact core microcosms (Fig. C-2 and C-3) reasonably well, indicating that continuous model is an appropriate substitution of weekly model. The weekly simulations for cDCE in Figures C-1c and C-2c stopped before day 300 because the exiting simulation already demonstrated the general trend and no more work was done to avoid the time-consuming and unnecessary work.
Figure C-1. Model predictions using the weekly approach and the continuous approach for (a) bromide, (b) TCE without reductive dechlorination, and (c) TCE and cDCE with reductive dechlorination.
Figure C-2. Weekly model predictions and data for (a) bromide, (b) TCE without reductive dechlorination, and (c) TCE and cDCE with reductive dechlorination.
Figure C-3. Continuous model predictions and data for (a) bromide, (b) TCE without reductive dechlorination, and (c) TCE and cDCE with reductive dechlorination.
C.3. Preliminary Modeling

Six scenarios (Table C-1) were simulated in preliminary modeling based on parameter in Table 4-1. All of the scenarios consider diffusion of bromide and TCE, as well as diffusion of cDCE if it was generated. Four of the scenarios included biotic reductive dechlorination of TCE to cDCE and four considered abiotic transformation of TCE and/or cDCE.

Table C-1. Six scenarios on potential processes in the intact rock core microcosms.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Bromide diffusion</th>
<th>TCE diffusion</th>
<th>TCE biotic</th>
<th>TCE abiotic</th>
<th>cDCE diffusion</th>
<th>cDCE abiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*An “x” indicates that the process was included in the scenario.*

The results are shown in Figures C-4 and C-5. In both figures, the panels on the left show the simulation results for TCE and cDCE and the panels on the right show the results for $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE. Simulation results for bromide are not shown because they are impacted only by diffusion and do not vary among different scenarios.

Scenario #1 simulates only matrix diffusion for TCE (Fig. C-4a) without any transformation process. TCE is predicted to increase inside the chamber and decrease in the core during the first 100 days. As diffusion reduces the concentration gradient between the core and the chamber, the rate of TCE entering the chamber decreases proportionally and is finally surpassed by the rate of sampling removal; consequently, the
concentration starts to decrease in the head chamber, and continues to decrease in the core.

The extent of enrichment during diffusion of $\delta^{13}$C-TCE was assumed to be minor (-0.2‰), based on results from Wanner et al. (174) (Table 4-1). This means that the diffusion rate for $^{12}$C-TCE is 0.2‰ faster than for $^{13}$C-TCE. Therefore, $^{13}$C-TCE becomes enriched in the core and depleted in the head chamber, compared to the initial $\delta^{13}$C-TCE level of -28.4‰ (Fig. C-4b). Due to the small enrichment factor, the extent of change was minor (i.e., less than 0.1‰).

Scenario #2 assumes that TCE is subjected to diffusion and reductive dechlorination to cDCE, and cDCE subsequently underwent diffusion but no further degradation. During the 35-day lag period prior to the onset of reductive dechlorination, only diffusion of TCE occurs, so the initial trend for TCE in Figure C-4c looks similar to the initial trend for scenario #1 (Fig. C-4a). After this lag, TCE is rapidly reduced to cDCE in both the chamber and the core. Although TCE reduction to cDCE is stoichiometric, the molar concentration of cDCE is higher than for TCE in the head chamber but lower than TCE in the core (Fig. C-4c) because cDCE has a higher diffusivity than TCE (Eq. 4.2; Table 4-1). Thus, while TCE is actively reduced to cDCE, a significant amount of cDCE diffuses from the core to the head chamber and exceeds the level of TCE.

$\delta^{13}$C-TCE is enriched during reductive dechlorination with an enrichment factor of -15‰ (Table 4-1). Due the fast reaction rate, $\delta^{13}$C-TCE rapidly increases from -28.4‰ to over zero within 100 days (Fig. C-4d, data not shown in full since the x-axis is zoomed
in for better visualization). The starting point for $\delta^{13}$C-cDCE is calculated as $(-28.4\%) + (-15\%) = -43\%$. As more TCE is reduced to cDCE, $\delta^{13}$C-cDCE approaches the original $\delta^{13}$C-TCE level $(-28.4\%)$. After TCE is consumed, enrichment of $\delta^{13}$C-cDCE continues at a much slower rate. This minor level of enrichment is driven by the fact that $\delta^{12}$C-cDCE diffuses into the chamber faster than $\delta^{13}$C-cDCE and gets removed faster during sampling.

For scenario #3, it is assumed that TCE undergoes diffusion and abiotic transformation at a rate of $1.6E^{-9}$ s$^{-1}$. The profile for TCE (Fig. C-4d) is similar to scenario #1, since the magnitude of abiotic transformation is small. The products of abiotic transformation (e.g., acetylene, CO$_2$ and NSR) are not shown. On the contrary, the profile for $\delta^{13}$C-TCE (Fig. C-4f) looks very different from scenario #1, because a large enrichment factor is assumed for abiotic transformation $(-25\%)$. Even though transformation only causes a small drop in TCE in the head chamber compared to scenario #1 ($\sim 0.4$ mg/L), $\delta^{13}$C-TCE in the head chamber enriches by more than 10\%.

For scenario #4, it is assumed that TCE undergoes biotic and abiotic degradation while cDCE does not undergo any degradation. The trend for TCE in Figure C-5a is similar to that in scenario #2 (Fig. C-4c), except that cDCE levels are lower in both the chamber and the core, since some of the TCE is abiotically transformed into other daughter products. The trends for $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE for scenarios #4 (Fig. C-5b) and #2 (Fig. C-4d) are also similar except that in scenario #4, $\delta^{13}$C-TCE enrichment starts prior to the 35-day lag time of reductive dechlorination due to the earlier onset of abiotic
transformation, and consequently $\delta^{13}C$-cDCE is approximately 0.5‰ higher than in scenario #2.

For scenario #5, it is assumed that TCE undergoes biotic and abiotic degradation, and cDCE undergoes abiotic degradation. The trends for TCE and cDCE (Fig. C-5c) are similar to those for scenarios #2 and #4, except that the concentration of cDCE in #5 is slightly lower. Due to the large enrichment factor for abiotic transformation of cDCE (-25‰), a notable increase in the $\delta^{13}C$-cDCE (Fig. C-5d) is observed, distinctive from scenarios #4 and #2.

For scenario #6, it is assumed that TCE undergoes only biotic degradation and cDCE undergoes only abiotic degradation. The trends for TCE and cDCE (Fig. C-5e) are similar to those for scenarios #2, #4, and #5. As indicated above, this is because the abiotic transformation rate is slow enough that the change in total concentration is not readily apparent. However, due to the appreciable enrichment factors for abiotic degradation and reductive dechlorination, there are notable differences in the trends for $\delta^{13}C$ (Fig. C-5f). $\delta^{13}C$-TCE remains unchanged prior to the onset of reductive dechlorination while $\delta^{13}C$-cDCE enriches at a high rate compared to the scenarios without abiotic degradation of cDCE.
Figure C-4. Preliminary modeling results for scenario #1 as: (a) TCE model and (b) δ\textsuperscript{13}C-TCE model; scenario #2 as: (c) TCE and cDCE model and (d) δ\textsuperscript{13}C-TCE and δ\textsuperscript{13}C-cDCE model; and scenario #3 as: (e) TCE model and (f) δ\textsuperscript{13}C-TCE model. In panels (b), (d) and (f), the horizontal black dashed lines represent the original δ\textsuperscript{13}C level, to facilitate comparisons to the model.
Figure C-5. Preliminary modeling results for scenario #4 as: (a) TCE and cDCE model and (b) $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE model; scenario #5, as (c) TCE and cDCE model and (d) $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE model; and scenario #6 as: (e) TCE and cDCE model and (f) $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE model. In panels (b), (d) and (f), the horizontal black dashed lines represent the original $\delta^{13}$C level, the green and orange dashed lines represent the slope of $\delta^{13}$C-TCE and $\delta^{13}$C-cDCE, respectively, to facilitate comparisons to the model.
C.4.  **Modeling Verification and Calibration**

Additional plots of simulation results to Figures 4-2 and 4-3 are shown in Figures C-6 to C-14. Panel (a) presents the results for bromide; panel (b) for TCE and cDCE; and panel (c) for $\delta^{13}C$, except for L1 and U1, where concentrations of TCE or cDCE were too low for $\delta^{13}C$ analysis. Simulations for the average concentration of TCE, cDCE and $\delta^{13}C$ within the cores are also shown in panels (b) and (c), as shown by the dashed lines.
Figure C-6. COMSOL model fit for (a) bromide and (b) TCE in the intact rock core microcosm U1. No isotope data were available for modeling.
Figure C-7. COMSOL model fit of (a) bromide and (b) TCE and cDCE for intact rock core microcosm L1. No isotope data were available for modeling.
Figure C-8. COMSOL model fit of (a) bromide, (b) TCE and cDCE, and (c) δ¹³C for intact rock core microcosm U2.
Figure C-9. COMSOL model fit of (a) bromide, (b) TCE and cDCE, and (c) $\delta^{13}$C for intact rock core microcosm L2.
Figure C-10. COMSOL model fit of (a) bromide, (b) TCE and (c) δ¹³C-TCE for intact rock core microcosm U3.
Figure C-11. COMSOL model fit of (a) bromide, (b) TCE and (c) $\delta^{13}$C for intact rock core microcosm L3.
Figure C-12. COMSOL model fit of (a) bromide, (b) TCE and cDCE, and (c) δ¹³C for intact rock core microcosm L4.
Figure C-13. COMSOL model fit of (a) bromide, (b) TCE and (c) δ¹³C for intact rock core microcosm U6.
Figure C-14. COMSOL model fit of (a) bromide, (b) TCE and cDCE, and (c) δ^{13}C for intact rock core microcosm L6.
C.5. **Comparison on Rate Constants**

A Summary of abiotic transformation enrichment factors ($\varepsilon$), abiotic transformation rate coefficients ($k$), and half-lives ($t_{1/2}$) for TCE and cDCE is provided in Table C-2. It listed values obtained from literature, crushed rock microcosms, and intact core microcosms. The abiotic enrichment factors used in the modeling ($\varepsilon_3$) are within the range reported in previous studies, as shown in Table C-2. However, it is possible that the degree of enrichment in rock at this site is outside this range.
Table C-2. Summary of $\varepsilon$, $k$ and $t_{1/2}$ from literature (A), crushed rock microcosms (B), and intact core microcosms (C).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
<th>Data</th>
<th>cDCE</th>
<th></th>
<th></th>
<th>TCE</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>$\varepsilon_0$</td>
<td>A</td>
<td>Table 1-1 to 1-3</td>
<td>-</td>
<td>-4.9</td>
<td>-23.5</td>
<td>-</td>
<td>-7.1</td>
<td>-51</td>
</tr>
<tr>
<td>$\varepsilon_1$</td>
<td>B</td>
<td>Identified $^{14}$C and $\delta^{13}$C</td>
<td>-75</td>
<td>-51</td>
<td>-99</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\varepsilon_2$</td>
<td>B</td>
<td>$^{14}$CO$_2$ + $^{14}$C-NSR and $\delta^{13}$C</td>
<td>-68</td>
<td>-48</td>
<td>-88</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\varepsilon_3$</td>
<td>C</td>
<td>$\delta^{13}$C model fit</td>
<td>-20</td>
<td>-</td>
<td>-</td>
<td>-25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$k$ (yr$^{-1}$)</td>
<td>A</td>
<td>Reference (116, 134)</td>
<td>-</td>
<td>6.6</td>
<td>10.8</td>
<td>-</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>$k$ (yr$^{-1}$)</td>
<td>B</td>
<td>$^{14}$CO$_2$ + $^{14}$C-NSR</td>
<td>0.04</td>
<td>0.02</td>
<td>0.07</td>
<td>0.04</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>$k$ (yr$^{-1}$)</td>
<td>C</td>
<td>$\delta^{13}$C model fit</td>
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<td>0.011</td>
<td>0.019</td>
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<td>0.008</td>
<td>0.019</td>
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<tr>
<td>$t_{1/2}$ (yr)$^b$</td>
<td>B</td>
<td>$^{14}$CO$_2$ + $^{14}$C-NSR</td>
<td>16</td>
<td>11</td>
<td>31</td>
<td>18</td>
<td>14</td>
<td>25</td>
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<tr>
<td>$t_{1/2}$ (yr)$^c$</td>
<td>B</td>
<td>$\delta^{13}$C and $\varepsilon_0$</td>
<td>-</td>
<td>4.8</td>
<td>7.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$t_{1/2}$ (yr)$^c$</td>
<td>C</td>
<td>$\delta^{13}$C model fit</td>
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<td>37</td>
<td>63</td>
<td>-</td>
<td>37</td>
<td>88</td>
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<td>$t_{1/2}$ (yr)$^c$</td>
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<td>$\delta^{13}$C and $\varepsilon_0$</td>
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<td>$t_{1/2}$ (yr)$^c$</td>
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<td>$\delta^{13}$C and $\varepsilon_1$</td>
<td>31</td>
<td>21</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$t_{1/2}$ (yr)$^c$</td>
<td>C</td>
<td>$\delta^{13}$C and $\varepsilon_2$</td>
<td>29</td>
<td>20</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

$^a$ Minimum and maximum values are based on the 95% confidence interval.

$^b$ Half-lives were calculated according to: $t_{1/2} = \frac{\ln (2)}{k} = -t \frac{\ln (C/C_0)}{\ln (C/C_0)}$

$^c$ Half-lives were calculated according to: $t_{1/2} = \frac{\ln (2)}{k} = \ln (2) \cdot \varepsilon \frac{dt}{d(\delta^{13}C)}$
**C.6. Sensitivity analysis**

Sensitivity analysis was performed on sampling removal rate, microbial motility, chemical diffusivities, abiotic transformation rates, enrichment factors, reaction retardation factor, and Monod kinetics, as shown in Figures C-15 to C-19.

A summary of the impact of different parameters on each dataset is provided in Table C-3.

**Table C-3. Impact of different parameters on data fitting.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bromide</td>
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<tr>
<td>chemical diffusivities ($\kappa$)</td>
<td>xx</td>
</tr>
<tr>
<td>sampling removal rate ($k_{chamber}$)</td>
<td>xx</td>
</tr>
<tr>
<td>half saturation coefficient ($K_S$)</td>
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</tr>
<tr>
<td>maximum specific growth rate ($\mu_{max}$)</td>
<td>o</td>
</tr>
<tr>
<td>bacterial decay coefficient ($d_H$)</td>
<td>o</td>
</tr>
<tr>
<td>reaction retardation factor ($R$)</td>
<td>o</td>
</tr>
<tr>
<td>abiotic transformation rate constants ($k$)</td>
<td>o</td>
</tr>
<tr>
<td>biotic and abiotic enrichment factors ($\epsilon$)</td>
<td>o</td>
</tr>
<tr>
<td>yield ($Y$)</td>
<td>o</td>
</tr>
<tr>
<td>microbial motility ($\lambda_e$)</td>
<td>o</td>
</tr>
</tbody>
</table>

*xx: significant impact                x: minor impact       o: no influence*
Figure C-15. Sensitivity Analysis of (a and b) porosity (dimensionless), (c and d) sampling removal rate (s⁻¹), and (e and f) microbial motility (m²/s).
Figure C-16. Sensitivity Analysis of (a and b) diffusivity (m²/s), (c and d) abiotic enrichment factor (‰), and (e and f) abiotic transformation rate constant (s⁻¹). All parameters pertained to TCE.
Figure C-17. Sensitivity Analysis of (a and b) diffusivity (m$^2$/s), (c and d) abiotic enrichment factor (‰), and (e and f) abiotic transformation rate constant (s$^{-1}$). All parameters pertain to cDCE.
Figure C-18. Sensitivity Analysis of (a and b) maximum specific growth rate \( (s^{-1}) \), (c and d) bacterial decay coefficient \( (s^{-1}) \), and (e and f) yield (copies/mg).
Figure C-19. Sensitivity Analysis of (a and b) TCE half saturation coefficient (mg/L), (c and d) TCE biotic enrichment factor (‰), and (e and f) reaction retardation factor in matrix (dimensionless).
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